PERIODICAL ROOM
RECEIVED
APR 26 1916
UNIVERSITY OF MICHIGAN
LIBRARY

VOLUME 17 NUMBER 4

THE ARCHIVES OF INTERNAL MEDICINE

EDITORIAL BOARD

JOSEPH L. MILLER, Chicago

RICHARD C. CABOT, Boston — THEO. C. JANEWAY, Baltimore

GEORGE DOCK, St. Louis — WARFIELD T. LONGCOPE, New York City

W. S. THAYER, Baltimore

APRIL 15, 1916

CHICAGO
AMERICAN MEDICAL ASSOCIATION
PUBLISHERS

Entered at Second-Class Matter, October 28, 1908, at the Postoffice at Chicago, Illinois, Under Act of Congress of March 3, 1879

CONTENTS OF VOLUME XVII

NUMBER 1

Observations on the Origin and Nature of the So-Called "Arteriosclerotic" White Blood Cell. Frank M. Sladen, M.D., Baltimore 1

Adams-Stokes Syndrome: Permanent Bradycardia Following Both Auricles and Ventricles. Successful Prolongation of the As-Vs Interval. W. S. Thayer, M.D., Baltimore 13

Tuberculin Quotient. Preliminary Studies of the Cutaneous Test of Tuberoid Immunity. Maurice S. Kleiner, San Francisco 21

The Specific Pathology and Pathogenesis of Anemia and Allied Conditions. A Physiological Method of Blood Regeneration. J. P. Schneider, M.D., Minneapolis 32

Blood Urea Determinations [illegible]. Harold Schwartz, M.D., and Caroline Moore, [illegible] 47

The Effect of Continuous [illegible] Series of Reciprocating Arrhythmia. William E. Simmons, M.D., [illegible] and Emanuel J. Meyer, M.D., Chicago 76

Non-Surgical Treatment of [illegible] of the Cervical Nervous System, According to the Principles of Swift and Ellis. Richard Skeeles, M.D., and Clyde L. Cummer, M.D., Cleveland 92

Proteolysis and Antiproteolysis [illegible] in the Coagulation of Blood. George R. Minot, [illegible] Francis P. Denny, M.D., Boston, and Daniel Davis, M.D., [illegible] 128

Clinical, Physiological and Pharmacological Observations on Psoralea Corylifolia from "Cheraita." M. A. Rothschild, M.D., G. E. Harmon, M.D., and [illegible] J. Daniels, M.D., Cleveland 140

Experiments on Sero-Agglutinins Using Bacillus Sanguis. A. Epstein, M.D., [illegible] 159

Diabetic Coma Occurring in a Boy Suffering from Chronic Pulmonary Tuberculosis Treated with Erythrema Antitoxin. Alfred Gordon, M.D., New York 175

NUMBER 2

The Nonparasitic Cerebral [illegible]. Alfred Gordon, M.D., and Max Kahn, M.D., Ph.D., Pittsburgh 181

Experimental Alcoholic Cirrhosis of the Liver. Addison L. Goven, M.D., Iowa City, Ia. 205

Sepsis and the [illegible] Thrombophlebitis and Other Thrombotic Diseases. [illegible], M.D., New York 209

Observations of the Lungs [illegible] Report of a Case. Robert Bishop, [illegible], M.D., Baltimore 221

Studies of the Liver [illegible] of Chloroform in an Animal. H. R. Wilson, [illegible] L. Richardson, M.D., Cleveland 230

Chemical Study of Epinephrine. H. Rainford Rowntree, Ph.D., Chicago 241

Observations in [illegible] Comparative Value of Clinical [illegible] Determination of Urine and Blood. Olga E. [illegible], [illegible] 270

Permanganate [illegible] W. G. [illegible], M.D., [illegible] 280

Treatment of [illegible] Multiple Sensitiveness. Report of a Typical Desensitization [illegible], M.D., Baltimore, Maryland, and Robert T. Cheney, [illegible] 288

Desensitization of [illegible] Hay-Fever. C. Mosenthal and Abraham [illegible], Philadelphia 299

Review: Diagnosis [illegible] 307

The Archives of Internal Medicine

Vol. XVII APRIL, 1916 No. 4

STUDIES OF EDEMA IN PNEUMONIA*

MARY E. MAVER AND A. B. SCHWARTZ
CHICAGO

It has long been known that acute infectious diseases may be accompanied by edema of the skin and subcutaneous tissues. While in some cases of pneumonia this edema may be sufficiently well marked to cause pitting on pressure, in the majority of cases, such evidence is not present, notwithstanding definite water retention. It would be expected, following the explanation of Fischer,[1] that edema would be an almost constant accompaniment of pneumonia, for we have here a disease in which several factors,[2] as the increase in ammonia output, the decreased carbon dioxid content of the blood, etc., make it very probable that there is an interference with oxidative processes of the organism.

According to Fischer the inhibition of oxidation, with the concomitant increase in acidity, is of great importance in the production of edema. That this edema may occur and not be appreciated is not surprising, when we remember the great amount of water retention which may occur before it becomes manifest as palpable edema.

By means of Schade's elastometer,[3] an instrument described in a previous communication, the question of edema in pneumonia has been studied. With this instrument it is possible to detect edemas not appreciable by the palpating finger. In a series of ten cases of pneumonia we have found with the elastometer the presence of slight or moderate edema in every case.

This edema, shown in the elastometric reading by the character of the curve and the deficient return of the curve to the base line, seems to be most marked during the height of the disease.

After the crisis, the curve has a tendency to return very slowly to normal. Occasionally, this return to normal is nearly complete soon

* Submitted for publication Dec. 11, 1915.

* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.

1. Fischer, Martin H.: Edema and Nephritis, New York, 1915.
2. Peabody, Francis W.: Jour. Exper. Med., 1912, xvi, 701.
3. Schade, H.: Ztschr. f. exper. Path. u. Therap., 1912, xi, 369.

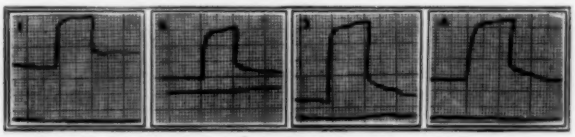


Fig. 1.—Curve 1 is a reading taken twelve hours after crisis. The subsequent readings illustrate the gradual but complete return to normal elasticity, the last reading being taken two weeks after crisis.

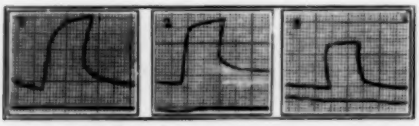


Fig. 2.—Curve 1, taken just before crisis, shows a very slight loss of elasticity. The second reading shows a greater loss. The last reading shows a complete return to normal.

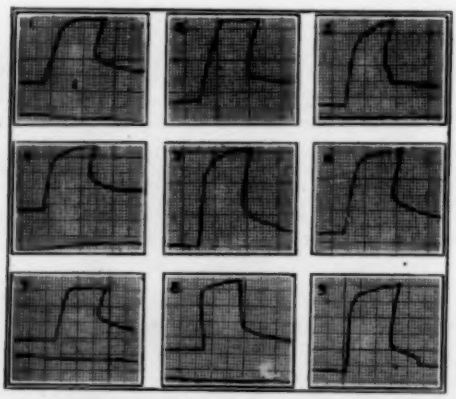


Fig. 3.—This figure illustrates the marked changes in tissue turgor which may occur in young children, following the infectious diseases. The patient on whom these readings were taken, was admitted to the hospital on the fifth day of a lobar pneumonia complicated by a double suppurative otitis media. Curve 1 is a reading taken on the day of admission. Curves 2 and 3 were taken during the height of the fever. The last six readings were taken during convalescence, the patient remaining in the hospital pending the selection of a suitable home by the Social Service Department. Clinically the only apparent delay in convalescence was a very noticeable lack of energy in the patient. The pronounced loss of elasticity and its persistent character is quite marked, the elasticity curve approximating normal six weeks after crisis.

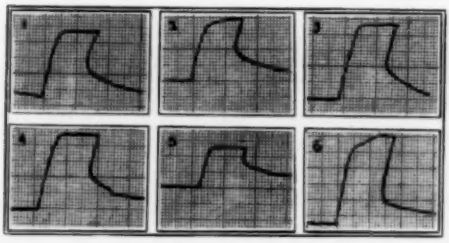


Fig. 4.—The readings in this case are further illustrations of the changes in elasticity which occur in young children. The patient was 3 years old. The first two readings were taken during the height of the disease. Curve 3 on the day of crisis. The elasticity loss persists after crisis.

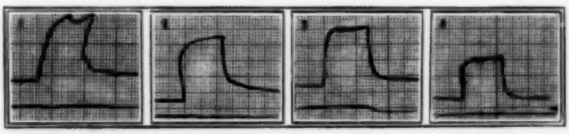


Fig. 5.—This figure shows a series of readings exhibiting a complete return to normal. The first two curves were obtained before crisis, the last two after crisis.

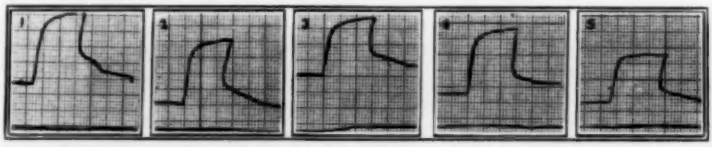


Fig. 6.—This shows variations in the elasticity curve taken during the course of a lobar pneumonia, complicated by an otitis media and furunculosis of the auditory canal. The clinical chart of the patient showed a similar irregularity in the temperature curve, which may bear some relationship to the fluctuations noted.

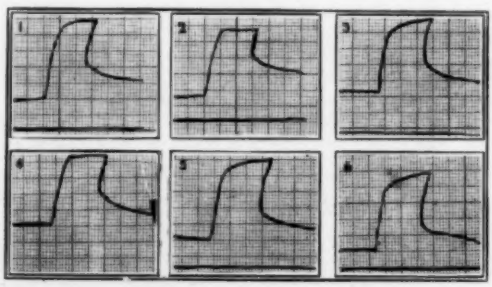
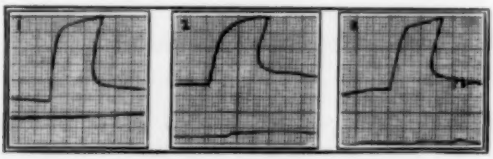
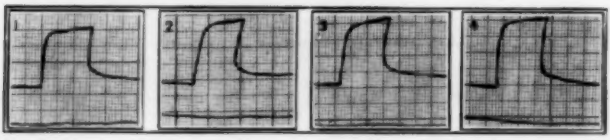


Fig. 7.—This illustrates an improvement in the elasticity curve immediately following crisis, the successive curves showing a gradual return to normal. The first two readings were taken before crisis, Curves 3 to 6 after crisis.

after crisis, while in other cases, there persists slight elasticity loss long after apparent convalescence. In some instances this persistent loss may be associated with complications. What factors influence such deficient return to normal, and their significance, we are not able, at present, to say.





Figs. 8 and 9.—Further examples of slow improvement after crisis. The first curve in each of these figures was taken before crisis, the other curves after crisis had occurred.

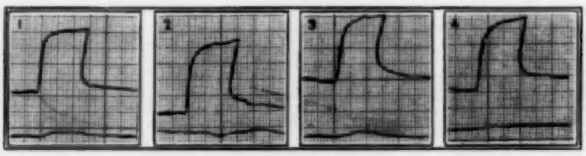


Fig. 10.—Curves 1 and 2 in this figure taken during the height of a lobar pneumonia show a slight loss of elasticity. On May 6, the temperature had reached normal by lysis. The reading taken at this time shows practically normal elasticity. Six days later, a reading shows a greater loss of elasticity than had been noted even during the height of the fever. Twelve hours after this reading was taken, the patient exhibited a marked characteristic rash of scarlet fever. Though further readings were not taken, on account of the necessary isolation of the patient, the striking change in the character of the curve suggests the possibility that associated with certain acute infectious diseases, earlier and profounder changes in tissue turgor may occur than has been heretofore thought.

The elastometric curves given in the text were all obtained at the wrist in patients admitted to the Children's Memorial Hospital with lobar pneumonia. In none of these cases was there any palpable edema. On account of the difficulty of working with children, some of the curves are not perfect. Nevertheless, the variations noted are sufficiently well marked for our study.

THE RELATION OF CHLORIDS TO EDEMA IN PNEUMONIA

The retention of chlorids in pneumonia has been studied by many observers, and recently, McLean,[4] using his new method of chlorin determination,[5] has shown that failure to excrete chlorin in pneumonia is associated with a lowered concentration of chlorids in the plasma, and that excretion begins at the time this concentration increases. That this increase is usually more marked than can be accounted for by the increased diet, has been noted by various observers. Numerous studies have been made to determine the point at which this retained chlorid is stored.[6]

CHLORIN DETERMINATIONS IN URINE IN PNEUMONIA

Date	Day of Disease	Clinical Observations	Urinalysis				Chlorid Per Cent. Blood Serum	Number of Curve
			Cl., Per Cent.	Cl., Gm.	Sp. Gr.	Output in 24 Hrs., c.c.		
1915 1/24	7th	Temp. 102-106.5.....	0.28	0.7	1.020	250	0.58	
1/27	10th	Temp. 102-104.....	0.16	0.44	1.018	275	....	Fig. 3
2/ 2	16th	Third day normal.....	.....	....	.....	...	0.57	
2/18	22d	Condition normal.....	0.67	3.9	1.015	575	0.61	
2/15	7th	Temp. 102-104.....	0.024	0.08	1.023	335	0.53	
2/19	11th	Improving.....	.....	....	.....	...	0.53	Fig. 5
2/21	13th	Improving.....	1.28	6.0	.....	470	0.60	
3/ 6	5th	Temp. 102-104.....	0.09	1.04	1.018	150	0.54	
3/14	13th	Temp. 98-101.....	0.33	1.51	.....	457	0.57	Fig. 6
3/22	21st	Temp. 99-104.....	0.23	1.08	1.011	470	0.64	
2/27	6th	Temp. 103-104.....	Trace	....	1.010	475	0.51	
3/ 4	11th	Temp. 99-100.....	Trace	....	1.013	90	0.57	Fig. 7
3/10	17th	Condition normal.....	0.63	3.5	1.010	570	0.56	
4/18	6th	Temp. 102-104.....	0.2	1.4	1.015	700	0.52	
4/21	9th	Normal.....	0.59	1.9	1.018	330	0.57	Fig. 8
4/25	13th	Normal.....	1.0	5.6	1.020	560	0.61	
4/21	5th	Temp. 102-104.....	0.065	0.3	1.019	462	0.54	
4/25	9th	Temp. 98-99.5.....	0.04	0.07	1.015	185	0.55	Fig. 9
4/27	11th	Temp. 98-101.....	0.18	1.65	1.009	920	0.62	
5/ 6	20th	Normal.....	0.97	7.1	1.015	740	0.65	
4/30	5th	Temp. 102-108.....	0.1	0.35	1.013	320	0.53	
5/ 2	7th	Temp. 101-104.....	0.22	1.0	1.017	490	0.50	Fig. 10
5/ 7	12th	Normal*.....	0.07	0.47	1.013	675	0.51	

* Fever the next day, scarlet fever six days later.

4. McLean, Franklin C.: Jour. Exper. Med., 1915, xxii, 212; ibid, 366.
5. McLean, F. C., and Van Slyke, D. D.: Jour. Biol. Chem., 1915, xxi, 361.
6. Peabody, Francis W.: Jour. Exper. Med., 1913, xvii, 71.

Having invariably found the presence of some edema in pneumonia by means of the elastometer, we undertook to correlate, if possible, these findings with chlorid determinations of the blood and urine. The difficulty of obtaining blood and the comparative infrequency of uncomplicated lobar pneumonia in the children at our disposal, made the number of our observations small.

For the determinations of chlorids in the blood, McLean's method was used; for urine, Volhard or Dehn's modification of the Volhard method.

In twenty-two observations of the chlorids in the blood serum, made in seven cases of lobar pneumonia, our results confirm those of McLean regarding the diminished concentration of chlorin in the plasma during the height of the fever, with increased concentration after crisis.

Of all these patients, elastometric determinations are given in the text figures. While the increased concentration of NaCl in the blood occurs almost immediately after crisis, there does not seem to be a corresponding change in the elasticity of the tissues, as determined on the wrist by the elastometer. The return to a normal concentration of NaCl in the blood serum usually occurs long before the elasticity curve has reached a normal state.

HYPOGLYCEMIA AND PROGRESSIVE MUSCULAR DYSTROPHY *

R. H. McCRUDDEN, M.D., AND C. S. SARGENT, S.B.
BOSTON

We recently made urine and blood examinations in a typical case of progressive muscular dystrophy which was being studied clinically by Drs. Goldthwait and Spear of Boston. The clinical findings are to be made the subject of a separate report, but the chemical findings seem well worth recording, even though they represent the data from but one case, since, if confirmed in other cases, they constitute a distinct contribution to our knowledge of carbohydrate metabolism. They are, therefore, published in the hope that similar examinations will be made by any other investigators who may observe a case of this uncommon disease.

THE OBSERVATIONS

The patient was a man 33 years of age who showed no abnormalities except progressive muscular weakness. He was put on a constant diet; the urine was saved and examined quantitatively for calcium, magnesium, nitrogen, creatinin, creatin, uric acid, and ammonia; and the blood for glucose, creatinin, creatin, uric acid, cholesterin, and nonprotein nitrogen.

DIET: The patient was kept on a constant diet throughout the period during which specimens were taken. The diet was constant in calories, and in its nitrogen, fat, and carbohydrate content; and practically identical foodstuffs were given from day to day. In spite of this physiologic constancy, the food was served in a variety of forms to the patient. This constancy in composition combined with an appearance of variety constitutes a characteristic and very useful feature of our studies at this hospital. The results are obtained by cooperation of physician and dietitian; and we believe that an adoption of the principles applied will serve to clear up many dietetic difficulties. The principle to be recognized is that the physician must prescribe the diet, for he alone knows what is needed; but the physician rarely possesses the technical knowledge necessary for putting his diet into palatable form and for giving it variety; for this he must call on the dietitian. The relation of physician and dietitian in this respect resembles somewhat that of the physician and pharmacist with respect to medicines. For convenience of explanation, the complete carrying out of the process may be divided into four steps:

1. After deciding on the approximate diet to be given, preliminary observations of a day or two are made to find out the amount of food the patient can take and his dietetic idiosyncrasies.

2. A list of foodstuffs is then given to the dietitian from which a dietary of several days is to be made out. In this case the following list was prescribed for each twenty-four hours:

4 eggs (which may be replaced in part by cheese in the proportion of 1 ounce of cheese to 1 egg)
4 glasses of milk
600 gm. of potato (which may be replaced in part by bread, spaghetti or rice in the proportion of 600 gm. of potato = 240 gm. bread = 150 gm. rice)
60 gm. butter.

* Submitted for publication Dec. 18, 1915.
* Laboratories of the Robert Breck Brigham Hospital.

All these foodstuffs are fairly constant in composition and in caloric value, so that composition and caloric value can easily be calculated. In addition, once a day, a small amount of lettuce, cucumber, celery, and tomato, and a small amount of fruit, either raw or cooked, were allowed; these latter foodstuffs have very little caloric value, and contain but little protein, fat, and carbohydrate. Such a diet has the composition shown in Table 1.

TABLE 1.—Composition of Experimental Diet

	Grams	Protein	Fat	Carbohydrate	Calories
Four eggs	200	25	25	...	335
Four glasses milk	800	32	32	32	560
Potato	600	..	..	120	492
Butter	60	..	48	...	446
Total		57	105	152	1,833

3. The third step—a conference of physician and dietitian for examination and alteration of the first tentative dietary submitted by the dietitian—comes after the dietitian has studied the diet list. It sometimes happens that the diet prescribed can be improved in palatability or variety by slight alterations or additions which may or may not be permissible, depending on the exact purpose of the diet. In this particular case the diet was correct as first submitted.

4. The fourth step consists in the preparation of a dietary. For this patient we give three days' diet as examples in Tables 2, 3 and 4.

TABLE 2.—Sample Diet

Date			Eggs	Milk, c.c.	Potato, gm.	Bread, gm.	Butter, gm.
19	Breakfast	Baked potato	.....	.....	100		
		Toast, butter, milk	.....	200	.....	48	14
		Soft cooked egg	1				
	Dinner	Bread, butter, milk	.....	225	.....	48	14
		Escalloped spaghetti with egg	1	85	.....	.....	12
		Lettuce (dressed)	.....	.....	.....	.....	2 (oil)
		Ice cream	1	175			
	Supper	Soup	.....	200			
		Baked stuffed potato	¼	20	200	.....	14
		Bread, butter, milk	.....	225	.....	96	14
		Baked custard	¼	100			
		Grape fruit					
	Total for the day		4	1,200	200	132	70

Besides vegetables and fruits and small amounts of sugar, flour, condiments, etc.

TABLE 3.—SAMPLE DIET

Date			Eggs	Milk, c.c.	Potato, gm.	Bread, gm.	Butter, gm.
20	Breakfast	Toast, butter, milk	.....	200	.....	48	12
		Cream toast	.....	125	.....	24	7
		Omelette	1				
	Dinner	Asparagus soup	.....	200	.....	.....	7
		Potato salad	.....	.....	200	.....	2 (oil)
		Baked stuffed egg	1	75	.....	.....	7
		Bread, butter, milk	.....	200	.....	48	12
		Lemon soufflé	¾				
		Foam sauce	¼				
	Supper	Cream of potato soup	.....	200	100	.....	9
		Croutons	.....	.....	.....	32	
		Toast, butter, milk	.....	200	.....	24	14
		Dropped egg on toast	1	.....	.....	24	
		Cut fruit					
	Total for the day		4	1,200	300	200	70

Besides vegetables and fruits and small amounts of sugar, flour, condiments, etc.

TABLE 4.—SAMPLE DIET

Date			Eggs	Milk, c.c.	Potato, gm.	Bread, gm.	Butter, gm.
21	Breakfast	Toast, butter, milk	.....	200	.....	48	14
		Grape fruit					
		Scrambled eggs	1	20	.....	.....	7
	Dinner	Soup, toast	.....	200	.....	24	
		Baked potato	.....	.....	200		
		Egg with tomato salad	1	.....	.....	.....	2 (oil)
		Bread, butter, milk	.....	200	.....	24	12
		Ice cream	1	160			
	Supper	Soup	.....	200	.....	.....	7
		Potato soufflé	½	20	200	.....	7
		Bread, butter, milk	.....	200	.....	64	14
		Orange fluff	½				
	Total for the day		4	1,200	400	160	70

Besides vegetables and fruits and small amounts of sugar, flour, condiments, etc.

We intend to make this question of dietetics as carried out at the Robert B. Brigham Hospital the subject of a separate publication and will not, therefore, go into any more details here.

URINE: The greatest care was observed in collecting the twenty-four-hour quantities of urine. This is a difficult task. The number and kind of accidents and errors that can occur in quantitative urine collections can be appreciated only by one who has made a special study of this subject. It is our belief that the results of urine analysis offered in the literature without accompanying creatinin determinations as evidence of completeness of collection are of

little value. Belief in the good intentions and trustworthiness of the nurse cannot replace creatinin determinations. With the most expert assistance, and with the most rigid system, losses and unexplainable accidents will occur. Since we intend to make this whole question of urine collection the subject of a separate publication, we will not go into details here further than to say that three factors are essential:

1. Creatinin determinations.

2. The closest oversight possible, supplemented by complete detailed ward reports concerning all the happenings to the patient written and signed by the nurse in charge (such reports serve as the basis of an investigation and often give the clue to the cause of irregularities disclosed by creatinin determinations).

3. Hearty cooperation in instituting, modifying, and carrying out the whole régime, the facilities for which are unusually good at this hospital.

The patient was kept in a room about 20 feet from the laboratory. He was not allowed to leave the room and all visitors except the day and night nurse in charge excluded. The régime to be carried out was explained to the nurses both by myself and by the superintendent of nurses and minute typewritten directions given besides. Urine was collected from 7 a. m. to 7 a. m. in special bottles containing a small amount of chloroform and alcoholic thymol solution as preservative. The bottle was kept right beside the patient's bedside in a box that was not unsightly or conspicuous. The urine secreted during the night was voided at 6 a. m. and then the bladder completely emptied again at 7 a. m. In order to avoid losses at stool, the patient was instructed to void each time before emptying the bowels. He was asked each time by the nurse if he had remembered to void in advance and also if he had lost any urine while evacuating the bowels. Reports similar to the one in Table 5 were sent to the laboratory each day with the urine.

TABLE 5.—SAMPLE OF DAILY REPORT

Patient's Name, Mr. X. Date, Oct. 22, 7 a. m. to Oct. 23, 7 a. m.

Urine	Feces	Food	Extra	Nurse
		7:30 a. m., orange, egg on toast, butter and milk	No accident	
9 a. m.	No stool		No accident	
No urine	9:05 a. m.		No accident	
11:25	No stool		No accident	
12:10	No stool		No accident	
No urine	12:15		No accident	
		12:30 p. m., potato soup, cheese, salad, bread, butter, potato with rice with egg sauce		
3:45	No stool		No accident	
		5:30, celery soup, bread, butter, milk, tomato, macaroni		7:30 a. m. to 7:30 p. m. Miss A. E. B.
9:15 p. m.	No stool		No accident	7:30 p. m. to 7:30 a. m. Miss M. A. B.
3:15 a. m.	No stool		No accident	
6:15 a. m	No stool		No accident	
7:00 a. m.	No stool		No accident	

The methods of analysis used were the following:

URINE:

Creatinin: Folin's method, described in Journal of Biological Chemistry, 1914, xvii, 470.

Creatin: Folin's original method of heating the urine with HCl for three hours on the water bath and determining creatin plus creatinin.

Uric Acid: Folin and Denis's method described in Journal of Biological Chemistry, 1913, xiv, 97.

Total Nitrogen: Kjeldahl method.

Calcium Magnesium: McCrudden's method described in Journal of Biological Chemistry, 1911, x, 187.

BLOOD:

Nonprotein Nitrogen: Folin and Denis's method described in Journal of Biological Chemistry, 1912, xi, 529.

Creatinin and Creatin: Folin and Denis's method described in Journal of Biological Chemistry, 1913, xiii, 469.

Glucose: Lewis and Benedict's method described in Journal of Biological Chemistry, 1915, xx, 61, as modified by Myers and Fine in their pamphlet on Chemical Composition of the Blood in Health and Disease, New York, 1915.

Uric Acid: Folin and Denis's method, described in Journal of Biological Chemistry, 1913, xiii, 469.

Cholesterin: Authenrieth and Funk's method, described in München. med. Wchnschr., 1913, lx, 1243.

The urine and blood examinations were all made in duplicate, except the sugar determinations, which were made in triplicate.

THE FINDINGS

Urine Examinations.—The twenty-four-hour quantity of urine varied in amount from 920 to 1,450 c.c.; it was slightly acid to litmus paper. It was made up each day to 1,500 c.c., examined qualitatively for sugar, acetone, diacetic acid and albumin (these compounds were always absent); and quantitatively for creatinin, creatin, uric acid, ammonia, calcium, magnesium, and nitrogen. The results will be found in Table 6.

TABLE 6.—URINE EXAMINATIONS

Date	Volume, c.c.	Specific Gravity	Creatinin	Creatin	Uric Acid	Total Nitrogen	Ammonia Nitrogen	Calcium	Magnesium
1	1,230	1.020	1.508	0.050	0.388	12.72	0.284	0.383	0.092
2	1,020	1.025	1.483	0.283	0.447	13.64	0.240	0.404	0.100
3	920	1.029	1.503	0.316	0.447	14.03	0.270	0.411	0.099
4	1,300	1.023	1.481	0.509	0.417	15.30	.....	0.477	0.091
5	1,180	1.022	1.493	0.024	0.385	14.34			
6	1,230	1.023	1.486	0.578	0.445	13.89			
7	1,450	1.021	1.481	0.409	0.405	14.36			
8	1,100	1.027	1.474	0.448	0.451	13.05			

Creatinin: The creatinin in the urine averages 22.6 mg. per kilo body weight, which is normal.[1] It is very constant from day to day, showing how completely and carefully the urine was saved. The variations from day to day were unusually slight; they are ordinarily

1. In a case of pseudohypertrophic muscular dystrophy Spriggs (The Excretion of Creatinin in a Case of Pseudohypertrophic Muscular Dystrophy, Biochem. Ztschr., 1907, ii, 206) found low creatinin excretion.

greater than found in this case. But we should like to point out—as a result of abundant experience—that when the twenty-four-hour quantities of urine are completely collected and the diet is constant and free from creatinin-producing substances, the excretion of creatinin is very constant, the variations being not more than 0.02 or 0.03 gm. per day from day to day.

Creatin: Creatin is present in large quantities. This is abnormal. By this method of creatin determination, which, we are convinced, is more reliable than the newer methods that appear to show traces of creatin even in normal urine, normal urine shows no creatin whatever.

Ammonia: The ammonia is normal in amount. This is significant. Other conditions in which creatin has been found present are associated with high ammonia excretion. In this case the low ammonia and absence of acetone and diacetic acid shows that we are dealing with a case of creatin-urea not accompanied by acidosis.

The uric acid, total nitrogen, calcium, and magnesium show no marked abnormalities, though the ratio of calcium to magnesium is rather high.

Blood Examinations.—Blood examination showed the results set forth in Table 7.

TABLE 7.—BLOOD EXAMINATIONS

	Mg. Per 100 Gm.
Creatinin	1.43
Creatin	2.86
Nonprotein nitrogen	28.9
Uric acid	2.30
Glucose	0.073 per cent.
Cholesterin	0.050 to 0.144 per cent.

Glucose: The glucose in the blood is low. Methods for the accurate quantitative determination of glucose in small amounts of blood have been available within only a very short time. Examination by these methods shows that the normal amount of glucose in the blood varies within very narrow limits and Allen[2] has pointed out that approximately the normal percentage is stubbornly maintained throughout prolonged starvation, almost up to death. By the method we used the variations are from 0.09 to 0.11 per cent. according to Lewis and Benedict;[3] from 0.09 to 0.12 per cent. according to Myers

2. Allen: Glycosuria and Diabetes, 1913.
3. Lewis and Benedict: Jour. Biol. Chem., 1915, xx, 61.

and Fine.[4] Our figures show an average of 0.073 per cent. (0.0731, 0.0717, and 0.0747 per cent. in three determinations).

Cholesterin: The cholesterin is very low. Of twenty-five determinations of cholesterin which we made in various other diseases all but four showed between 0.19 and 0.26 per cent. The four exceptions showed 0.17, 0.17, 0.14, and 0.15 per cent., respectively.

The nonprotein nitrogen, uric acid, creatinin, and creatin content of the blood show no abnormalities.

DISCUSSION

The striking abnormalities were, then, a low glucose and cholesterin content of the blood, and the presence of creatin in the urine.

The *creatin* in the urine suggests some abnormality of the glucose metabolism. It is found in the urine in conditions like diabetes and starvation when sugar is not being properly oxidized, and in such cases is usually associated with acidosis. In the present instance the normal ammonia excretion and absence of acetone and diacetic acid from the urine show that we are dealing with creatinuria without acidosis.

The low *cholesterin* content of the blood has no definite significance in the present state of our knowledge of the physiology of this substance. The most that can be said is that it makes us think of the possibility of involvement of the adrenals, a possibility that is still further indicated by the hypoglycemia.

The association of muscular weakness with *hypoglycemia* is striking, for we know that the muscle gets its power through oxidation of sugar; and a low sugar content of the blood might be looked on as sufficient cause for muscular weakness. Many investigators have demonstrated the power of sugar to make exhausted muscles capable of more work; the subject, with literature, is discussed by Fürth and Schwarz.[5] A direct relationship between muscular weakness and hypoglycemia has been noted by different investigators. Weiland,[6] using a Gärtner ergostat, had several of his colleagues do severe muscular work, almost to the point of exhaustion, and determined the glucose content of the blood before and after the work. In every case there was a decrease in the glucose content of the blood, the average decrease being 27 per cent. In three cases of Addison's disease — a condition

4. Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915. The results by older methods are less to be relied on.

5. Fürth and Schwarz: Ueber die Steigerung der Leistiengsfähigkeit des Warmblütermuskels durch gerinnungsbefördernde Muskelgifte, Pfluger's Arch. f. d. ges. Physiol., 1909, cxxix, 525.

6. Weiland: Ueber den Einfluss ermüdender Muskelarbeit auf den Blutzuckergehalt, Arch. f. exper. Path. u. Pharmakol., 1907-08, xcii, 223.

characterized by intense muscular weakness—quantitative determinations of the glucose content of the blood by Porges[7] showed 0.052 per cent., 0.033 per cent., and 0.067 per cent.— all very low results. Porges followed this point further in experiments on animals. He removed the adrenals from several dogs and compared the glucose content before and a few hours after the operation. His figures are as shown in Table 8. In every case there was a decrease in the glucose content of

TABLE 8.—GLUCOSE CONTENT BEFORE AND AFTER REMOVAL OF ADRENALS

	Per Cent. Before	Per Cent. After
Dog 1	0.258 0.103	0.0058 0.0057
Dog 2	0.120	0.033
Dog 3	0.084	0.066
Dog 4	0.092	0.044

the blood. Mayer[8] had previously reported a fall in the blood sugar content in cats after removal of the adrenals. This association of fall in blood sugar content with muscular asthenia and decrease in adrenalin is quite in harmony with the fact previously observed by Batteli and Boatti[9] and by Schur and Wiesel[10] that the epinephrin content of the blood of dogs decreases when these animals are made to undergo exhausting work on a treadmill. An association between muscular asthenia and hypoglycemia has been noted in a case of dyspituitarism and in atrophied babies. In Cushing's[11] book on the hypophysis there is a case history of a man suffering from dyspituitarism, showing marked muscular asthenia and low blood sugar (0.039 and 0.053 per cent. in two determinations). Frank[12] observed in three atrophied babies, respectively, 0.046 per cent., 0.040 per cent., and 0.050 per cent. blood sugar (0.10 to 0.11 per cent. is the average figure for normal babies).

Muscular asthenia alone, secondary to some other condition, is not necessarily accompanied by hypoglycemia. Through the kindness of

7. Porges: Ueber Hypoglykämie bei Morbus Addison sowie bei nebennierenlosen Hunden, Ztschr. f. klin. Med., 1909-10, lxix, 341.

8. Mayer: Ablation des surrénales et diabète pancréatique, Compt. rend. Soc. de biol., 1908, lxiv, 219.

9. Battelli and Boatti: Influence de la fatique sur la quantité d'adrénaline existant dans les capsules surrénales, Compt. rend. Soc. de biol., 1902, liv, 1203.

10. Schur and Wiesel: Beiträge zur Physiologie und Pathologie des chromaffinen Gewebes, Wien. klin. Wchnschr., 1907, xx (2), 1202.

11. Cushing: The Pituitary Body and Its Disorders. Phila., 1910, p. 130.

12. Frank: Ueber einige Grundtatsachen aus der Physiologie des Blutzucker nebst methodisher Vorbemerkungen, Ztschr. f. physiol. Chem., 1910-11, lxx, 129.

Dr. Goldthwait we had an opportunity to examine the blood in two very rapidly progressing cases of chronic arthritis of the infectious type, one of them of less than twelve months' duration, the other of less than six months' duration. In both cases there was a very marked wasting of the muscles. Examination showed 0.119 per cent. and 0.120 per cent. blood sugar, respectively, in the two cases. The significant feature in these cases was not muscular weakness alone, but rather muscular wasting.

As to the cause of the low sugar content of the blood, two possibilities may be imagined:

1. Hypoglycemia can be brought about by a lowered threshold value for excretion of glucose through the kidneys — renal diabetes. Thus the sugar content of the blood can be lowered by the administration of phloridzin, a drug which increases the permeability of the kidney for glucose and causes glucosuria. With the patient we had under observation the fact that glucose was absent from the urine excludes this as a possible cause of the hypoglycemia.

2. Glucose is constantly passing from the blood into the muscles to undergo oxidation; this loss is made up by the introduction of glucose into the blood from the glycogen store in the liver; and hypoglycemia can be brought about as the result of loss of balance between the rate at which glucose is introduced into the blood and the rate at which it disappears from the blood into the muscles. This regulation of the rate of formation of glucose from glycogen is under the control of the adrenals, the pituitary body and the thyroid gland;[13] administration of these substances or stimulation which increases the amount of adrenal principle in the blood leads to hyperglycemia.[13] That the cause of hypoglycemia in Addison's disease and after removal of the adrenals is associated with a decreased formation of glucose from glycogen is evident from the experiments of Porges,[14] who showed that after removal of the adrenals from dogs the liver becomes practically free from glycogen. Porges[15] followed the subject still further and noted that adrenal principle increases not only the rate of formation of glucose from glycogen, but also the rate of formation of glycogen from glucose; so that when the adrenals are diseased the liver loses its power to store glycogen. Frank and Isaac,[16] furthermore, have shown that the hypoglycemia resulting from phosphorus poisoning results from a loss of power of the liver to store glycogen.

13. The literature on this subject is voluminous.

14. Porges: Ueber Hypoglykämie bei Morbus Addison sowie bei nebennierenlosen Hunden, Ztschr. f. klin. Med., 1909-10, lxix, 341.

15. Porges: Zur Pathologie des Morbus Addison. II. Ueber Glykogenschwund nach doppelseitiger Nebennierenextirpation bei Hunden, Ztschr. f. klin. Med., 1910, lxx, 243.

16. Frank and Isaac: Ueber das Wesen des gestörten Stoffwechsels bei der Phosphorvergiftung, Arch. f. exper. Path. u. Pharmakol., 1910, lxiv, 274.

The possibility that the low sugar content of the blood in our case might be due to inability to store glycogen as effectively as normally, led us to determine the glucose content of the blood after a short period of starvation. Normally, as fast as the glucose of the blood is oxidized, a continuous new supply resulting from glycogenolysis maintains the glucose of the blood at its normal level; starvation and ingestion of food has but little effect on the glucose content of the blood. Any interference with glycogen storage might become apparent by a fall of the glucose content of the blood after a short period of starvation. It was not our purpose really to starve the patient, but merely, by omitting a meal, to exclude the effect of food on the glucose content of the blood; the patient was asked merely to omit his breakfast on one day. He went without food from 6 p. m. one evening until noon the next day, when blood was again taken for examination. The glucose content was 0.064 per cent. (triplicate determinations showed 0.0651, 0.0628 and 0.0637 per cent., respectively) at this time — decidedly lower than when previously taken. The decrease is at least suggestive, though not of course to be taken as definite indication of any disturbance in glycogenolysis.

Another fact of interest to be mentioned here is the relationship between loss of power to store glycogen and fatty transformation. In one of his experiments on dogs Porges[15] noted that during the operation of removing the adrenals the liver was normal; at necropsy, shortly afterward, it was observed that the liver had undergone fatty transformation; in other words, fat was stored instead of glycogen. A similar observation is recorded by Frank and Isaac;[16] these investigators noted that the loss of power of the liver to store glycogen which results from phosphorus poisoning was accompanied by a storage of fat instead. The fact noted by Cushing[11] that fatty transformation of the liver cells accompanies states of hypopituitarism — another condition associated with muscular weakness and hypoglycemia — may be mentioned in this connection. In view of these facts showing a relationship between hypoglycemia, fat storage and loss of power to store glycogen, the significance of the deposition of fat in the muscle cells in cases of pseudohypertrophic muscular dystrophy becomes apparent; the fat storage may well be due to an inability of the muscle to exercise its normal function of storing glycogen, fat instead being stored.

TREATMENT

In view of the facts connecting hypoglycemia, muscular asthenia and diminished activity of the adrenals and hypophysis, Dr. Spear suggested the use of epinephrin and pituitary extract for treatment. A detailed report of the treatment and its results will be made elsewhere. The point to be brought out here is the relationship between improvement in the physical condition and the increase in blood sugar.

Improvement in health, strength and weight was prompt and marked; and, with the improvement, the sugar content of the blood rose first to 0.080 per cent. then to 0.099 per cent., the latter a normal amount. An increase in the cholesterin content of the blood accompanied the increase in sugar content; with 0.080 per cent. sugar the cholesterin content was 0.177 per cent; with 0.099 per cent. sugar the cholesterin content was 0.211 per cent., the latter a normal amount.

As a preliminary to leaving our care entirely and returning to his home, the patient was allowed to leave the hospital and do much as he pleased in the city. At the end of a week his condition showed no change; the blood sugar showed a slight drop to 0.087 per cent., a figure which is only slightly below the normal average.[17]

It may be wise here to warn for the present against any attempts to treat such conditions by intravenous administration of glucose, a form of treatment that might naturally suggest itself. Underhill[18] tried this form of treatment on three dogs which showed hypoglycemia and muscular asthenia as a result of hydrazin poisoning; the animals all died within a few hours.

SUMMARY

In a case of progressive muscular dystrophy, the creatinin excretion was normal; large amounts of creatin were found in the urine; the ammonia excretion was low; there was no evidence of acidosis; the amount of sugar in the blood was low; the cholesterin content of the blood was low. A relationship between sugar content of the blood and ingestion of food suggested the possibility of a decreased power to store glycogen. Treatment which increased the glucose and cholesterin content in the blood led to improvement in the clinical condition.

We intend to follow this subject further. But since this disease is not common, our present results are published in order to call attention to the desirability of determining blood sugar in other cases of a similar nature. If confirmed in other cases, the association of hypoglycemia and muscular asthenia in this disease is, of course, of great significance in the physiology of carbohydrate metabolism.

Robert Breck Brigham Hospital.

17. Two months after leaving us we received a letter from the patient, who is in a distant part of the country, stating that he had continued taking epinephrin and pituitary extract; that he had gained much in weight and strength, and that his muscles were larger and firmer.

18. Underhill: Studies in Carbohydrate Metabolism. 1. The Influence of Hydrazin upon the Organism with Special Reference to the Blood Sugar Content, Jour. Biol. Chem., 1911-12, x, 159.

A CLINICAL STUDY OF DELAYED GASTRIC EMPTYING

BASED ON 185 CONSECUTIVE CASES OCCURRING IN AN UNSELECTED SERIES OF 1,600 PATIENTS WITH DIGESTIVE SYMPTOMS *

I. H. LEVY, M.D.
Associate Professor of Clinical Medicine, College of Medicine, Syracuse University

AND

J. L. KANTOR, M.D.
SYRACUSE

I. OBJECT OF STUDY, MATERIAL, METHOD

The object of our study was to determine the general incidence, and, as far as possible, the actual causes of delayed emptying of the stomach as met with in general practice. For this purpose we reviewed the records of 1,600 patients complaining of disturbances of digestion. Not all of these were suffering from actual lesions of the digestive tract. Many patients, as might be expected, had their primary source of trouble in other systems, such as the pulmonary, cardiorenal, or nervous.

The diagnosis of delayed emptying was made from the evidence furnished by tube or Roentgen-ray examinations, or both. For the tube test, the patients were asked to eat an "ordinary dinner" of soup, potato, meat, bread and butter, and a simple dessert such as rice pudding. As substitutes for such a meal, where necessary, we employed a "breakfast" of eggs, toast and cereal, or a "light lunch" (omitting meat; otherwise the same as the dinner). A stomach residue obtained seven hours after the dinner,[1] six hours after the lunch, or five hours after the breakfast, was regarded as pathological. The actual choice of food was always left to the patient, our purpose being to have him select a meal that he would ordinarily (namely, when well) dispose of without symptoms. Moreover, the patient was simply instructed to

* Submitted for publication Jan. 12, 1916.

1. The reason for taking seven hours as the outer limit of the normal is, first, because it is known that the normal stomach digests food ordinarily considered difficult in less than six hours; and secondly, because our meals are so arranged that rarely over six hours elapses between them. The normal stomach is no doubt capable of considerable latitude in the quantity of food it can hold. The weak or sick stomach, on the other hand, objects to undertaking the digestion of the new meal before it has disposed of the old. The symptoms may be due either to the increased load or to the disturbed chemistry, the gastric secretions at the end of digestion being different from what they are at the beginning.

take the meal in question, and to present himself after the appropriate interval, not having partaken of food or drink in the meantime. The exact nature of the examination was usually not disclosed. Finally, as the tube had generally been passed at least twice before (aspiration of fasting stomach and of Ewald test breakfast), it is believed that the psychic factor was reduced to a minimum.

The Roentgen-ray diagnosis of the condition was made from a visible residue six hours after the ingestion of the Rieder meal. In our earlier cases we used 50 gm. of bismuth subcarbonate in 300 gm. of cereal. Subsequently we employed 100 gm. of barium sulphate in cereal or in 500 c.c. of buttermilk. In accordance with other workers we found the emptying time of all of these combinations to be practically the same under similar circumstances.

We believe that the Roentgen-ray investigation of gastric emptying should always be supplemented by the use of the stomach tube[2] after the administration of "natural" motor meals as suggested above. Since our adoption of this practice we have discovered the existence of delayed emptying in a surprisingly large number of individuals who got rid of their opaqe meal within the normal time limits. These cases, which numbered thirty in our series, or about 21 per cent. of the total, have been indicated by a star for more ready differentiation in the accompanying large table. To be sure they represent the milder forms of delay due generally to pyloric spasm (occasionally to atony of the stomach), and not to actual obstruction. Although of little interest to the surgeon they are of great importance to the patient, and their existence should be borne in mind by the student of this condition.

II. THE INCIDENCE OF DELAYED GASTRIC EMPTYING

General Incidence.—In our total of 1,600 cases presenting gastric symptoms, delayed emptying of the stomach occurred 185 times, a general incidence of 11.5 per cent.[3]

Sex Incidence.—The sex and age incidence were computed from a total of 898 gastro-intestinal cases examined roentgenologically. Of the 483 men in this group, 78, or 16.1 per cent., showed delay. Of the

2. Bassler (Diseases of the Stomach and Upper Alimentary Tract. Phila., F. A. Davis Co., 1913, p. 213) and Cole (Relation of Lesions of the Small Intestine to Disorders of the Stomach and Cap as Observed Roentgenologically, Am. Jour. Med. Sc., 1914, cxlviii, 92) have recommended the addition of an opaque substance to an "ordinary" meal, and employ only the Roentgen ray in this diagnosis.

3. For the purpose of more careful study and for the compilation of some of our data, we selected from the total 185 cases, 141 which were studied roentgenographically. These will be referred to hereafter as the "Roentgen-ray series." Protocols of these cases are given in tabular form at the end of the article (Table 2).

415 women, 63, or 15.1 per cent., were slow in emptying their stomachs. This slightly greater frequency in man is perhaps due to the higher incidence of duodenal ulcer.

Age Incidence.—Of 464 patients under 40 years, 62, or 13.3 per cent., showed delay, whereas of 252 men 40 or over, 48, or 19 per cent., incidence was 79, or 18.2 per cent. The distinctly greater frequency in later years is explained possibly by the practical limitation of cancer to that period of life. In addition, obstructing ulcers are more frequent as age advances.

Age and Sex.—The distribution of the delay cases in regard to both age and sex was as follows: Of 231 men under 40, 30, or 12.9 per cent., showed delay, whereas of 252 men 40 or over, 48, or 19 per cent., came under this head. Of 233 women under 40, 32, or 13.7 per cent., showed delay, whereas of 182 women 40 years or over, 31, or 17.6 per cent., were slow in emptying their stomachs. For some reason delayed gastric emptying seems to be more common in women than in men under 40 years, a finding not in accord with the generally higher incidence in the male sex.

III. THE CHIEF FACTORS INFLUENCING GASTRIC EMPTYING

The chief factors influencing gastric emptying may be enumerated categorically as follows:

1. Shape of stomach.
2. Character of meal.
3. Tone of stomach.
4. Acidity of gastric juice.
5. Nervous factors.
6. Mechanical obstruction.

Shape of Stomach.—As a general rule it is safe to say that the nearer the pylorus is to the lower pole, the sooner will the stomach get rid of its contents. Thus, a cow-horn stomach (pylorus at lowest point) will empty itself of the Rieder meal in two to three hours. As the fish-hook type is approached, the time lengthens to four or five hours. On the other hand, that a high placed pylorus (so-called water-trap stomach) will in itself cause pathological increase in the emptying time is, to say the least, not easy to prove. Theoretically, of course, it may be possible, and it has recently been claimed (Satterlee and LeWald[4]) that the water-trap stomach may be a rather prominent factor in producing delayed gastric emptying. We have carefully studied and measured over 350 plates with this point in view, but have been unable fully to confirm this finding. In only one of our 185 cases was a water-trap stomach the only pathological condition present, and

4. Satterlee, G. R., and LeWald, L. T.: One Hundred Cases of Water-Trap Stomach, Jour. Am. Med. Assn., 1913, lxi, 1340.

we are forced to conclude, therefore, that in most, if not all, cases of "hypomotility" associated with the peculiarity in question, the cause of the delay must be sought for in some other factor than in the abnormal shape of the organ.

Character of the Meal.—The amount, chemical nature, and physical form of the food selected, each play an important rôle in determining the emptying time. Thus, an opaque meal of the ordinary bulk leaves the stomach sooner than a full dinner; carbohydrates leave sooner than proteins or fats; fluids sooner than solids.

Tone of Stomach.—Normally the stomach "grasps" its contents firmly, and is seen roentgenographically to be filled to the cardia. This so-called peristaltic function of Stiller is impaired in muscular weakness (atony or myasthenia gastrica). The Roentgen-ray appearance in this condition is characteristic. The stomach is generally, though of course not necessarily, ptosed, the *Magenblase* long and pyriform; the pars media is the narrowest portion of the organ, the walls often being in contact. Both the transverse and vertical diameters through the incisura angularis are excessive. In contrast to the normal state, the stomach looks and fills like a sack. The peristalsis is generally, though not necessarily, diminished; the essential feature, however, is the inability of the organ to accommodate itself to the size of the load. Atony of the circular fibers, in which the stomach enlarges laterally instead of longitudinally (Todd[5]), is probably of less clinical importance than the type just described.

Atony is rightly regarded as a prominent cause for the inability of the stomach to propel its contents forward. Of 240 cases of atonic stomach, 73, or over 30 per cent., failed to expel the Rieder meal in the proper interval. Conversely, over one-half (54.6 per cent.) of the Roentgen-rayed cases of delayed emptying showed atony. In many instances (see Section IV) this was the most prominent, and very often the only, organic finding.

Acidity of Gastric Juice.—Although this factor might theoretically be grouped under the head of nervous influences, it seems better for practical purposes to consider it in a section by itself. Generally speaking, the less the acidity, the more rapid the emptying, and the higher the acidity, the slower the emptying. Thus, in "anacidity" we speak of a loss of the pyloric reflex, and in "hyperacidity," of a spasm of the pylorus.

In order to obtain a more precise idea of these relationships we calculated the incidence of delayed emptying in our "anacidity" cases

5. Todd, T. W.: The Clinical Anatomy of the Gastro-Intestinal Tract. New York, Longmans, Green & Co., 1915, p. 89.

on the one hand, and in our "hyperacidity" cases on the other, and compared these findings with the general incidence of delayed gastric emptying.

Of 154 "anacidities" 14, or 9 per cent., showed delay; of 748 "hyperacidities" 78, or 10.4 per cent., showed delay. However, the interfering influence of obstruction, at least, should be considered. Making a rough correction for this factor by subtracting from each group those cases showing the presence of food in the fasting stomach, the figures become 4.5 per cent. for the former, and 9.4 per cent. for the latter. Thus the incidence of delayed gastric emptying is twice as great in nonobstructive "hyperacidity" cases as it is in nonobstructed stomachs with "anacidity."

Nervous Factors.—The nervous system may influence the emptying of the stomach in two possible ways. The first and most important is by the closure of the pyloric sphincter in response to some irritant. In many, though by no means in all, cases, the expression of this irritant seems to be an "increased acidity" of the gastric juice. Clinically, spasm may arise directly from causes within the stomach, such as ulcer, or "reflexly" from causes without the stomach, as in disease of the gallbladder. Roentgenoscopically, pylorospasm is often associated with hyperperistalsis.

Another type of nervous disturbance connected with retarded propulsion is that which occurs in fatigue, emotional states, or intoxications. This is to be distinguished from the first type in that the cause is general or systemic and the pathological condition is probably one of entire inhibition of gastric activity. Thus, it has been shown that in frightened animals the secretory as well as the motor functions are held in abeyance. This point will be discussed further (Section V).

Mechanical Obstruction.—Foreign bodies, hair balls, for example, seem seldom to obstruct the lumen of the stomach sufficiently to cause delay in emptying. Within the wall of the stomach the most frequent causes are new growths or cicatrized or indurated ulcers at the pylorus. Edema of the gastric wall as a result of ulcer is a possible cause of delay, though clinically this cannot be distinguished from spasm. The chief extragastric factors are tumors or adhesions usually located in the neighborhood of the pylorus. These may of course act reflexly by interference with the nerve supply.

IV. THE IMMEDIATE OR ACTUAL CAUSES OF DELAYED GASTRIC EMPTYING

For all practical purposes, there are but three immediate causes of delayed gastric emptying. They are, in the order of their frequency, spasm, obstruction, and atony. Their respective incidence in our Roentgen-ray series was as follows:

	Cases	Per Cent.
Spasm	83	or 58.8
Obstruction	30	or 21.2
Atony	24	or 17.0
Undetermined	4	or 2.8

Naturally, two or even all three of these causes may be present at the same time. In computing the foregoing figures only the most prominent cause was used in each instance.

V. DISEASES PREDISPOSING TO DELAYED GASTRIC EMPTYING

The clinical conditions met with, and perhaps responsible for, the delayed gastric emptying in our entire series of 185 cases, are presented in the accompanying table (Table 1). Unfortunately, we were unable to control these findings in many instances by repeating the tests after the presumptive cause was removed. Although the individual diagnosis may be open to objection, we feel that our conclusions in general are as nearly accurate as work done under the conditions of ordinary practice will permit. It should be mentioned also that the groups into which our cases are divided are not necessarily mutually exclusive. Thus, many of the patients presented more than one of the seventeen conditions or diseases considered in the table. For the sake of simplicity, however, only the most prominent condition was considered in each case in working up the material.

TABLE 1.—CLINICAL DIAGNOSIS IN 185 CASES OF DELAYED GASTRIC EMPTYING

Disease	Cases	Per Cent.	Disease	Cases	Per Cent.
1. Ulcer	75	40.5	10. Chronic appendicitis	2	1.0
2. Diagnosis not clear	25	13.5	11. After gastro-enterostomy	2	1.0
3. Unexplained high acidity	17	9.1	12. Extragastric pressure	2	1.0
4. Autointoxication (including migraine)	14	7.5	13. Carcinoma of colon	1	0.5
5. Carcinoma of stomach	14	7.5	14. Mucous colitis	1	0.5
6. Ptosis with atony	13	7.0	15. "Constipation"	1	0.5
7. Cholelithiasis	7	3.7	16. Nephrolithiasis (?)	1	0.5
8. Adhesions (intestinal)	5	2.7	17. Apprehension neurosis	1	0.5
9. Tuberculosis (systemic, including pulmonary)	4	2.1			

Gastroduodenal ulcers seem to be responsible for about half the cases of delayed emptying. Our incidence of 40.5 per cent. represents a conservative estimate, since all doubtful cases were thrown into one of the two following groups: A positive diagnosis of duodenal ulcer was made in thirty-eight cases, or in 50.6 per cent. of the series. Obstructing ulcers at the pylorus (food in fasting stomach) occurred

twenty-five times, or in 33.3 per cent. of the cases. Thus a reasonably certain diagnosis of the situation of the ulcer was made in 84 per cent. of the cases.

Cases of autointoxication with so-called bilious or migraine attacks formed a fairly large group in the series. Although well known to special writers on the subject (an interesting chemical study of a case is reported by Harley and Goodbody[6]), the frequency of the association of this condition with delayed emptying of the stomach is perhaps not fully enough appreciated. The relief obtained by many of these patients from vomiting is a striking illustration of the intimacy of this relationship. Just what this relationship is as regards cause and effect, we are not able to say. All of our cases happened to be examined in the interval between attacks.

The carcinoma cases were of the obstructing type situated at the pylorus. The colloid variety, which can be diagnosed readily, and in most cases only, by the Roentgen ray, is of course associated with gastric "hypermotility."

The group designated "ptosis and atony" is of great interest. It includes those cases of "asthenia universalis" so well described by Stiller.[7] The recognition and correction of both the underlying conditions completely change the outlook for many of these sufferers. One of the women in this category was lactating at the time of the examination. It is interesting to speculate how much this state had to do with the gastric delay. As long ago as 1883, v. Leube[8] pointed out that menstruation was in itself likely to increase the emptying time of the stomach.

In two of the patients operated on for cholelithiasis a large, tense gallbladder was found. It is possible that the delay may have been due to actual pressure as well as to reflex spasm.

The tuberculosis cases were made up as follows: Three were pulmonary, two, strange to say, with achylia; the fourth was a case of bone tuberculosis. A fifth case, with gastric ulcer, had lesions of the kidney and testicle. If the "hypomotility" in these cases is really due to the tuberculosis it may be assumed that the delay is brought about by a severe systemic intoxication. This may therefore be an illustration of the second type of nervous disturbance described in Section III.

Chronic appendicitis was not often met with in our series. We made a positive diagnosis in but two cases (1 per cent.), a rather low inci-

6. Harley, V., and Goodbody, F. W.: The Chemical Investigation of Gastric and Intestinal Diseases. London, E. Arnold, 1906, p. 83.

7. Stiller, B.: Enteroptose im Lichte eines neuen Stigma neurasthenicum, Arch. f. Verdauungskr., 1896, ii, 285.

8. Von Leube, W.: Beiträge zur Diagnostik der Magenkrankheiten, Deutsch. Arch. f. klin. Med., 1883, xxxiii, 8.

dence, but apparently in keeping with the findings of Carman and Miller.[9]

A further comparison of our findings with those of the Mayo Clinic may be made from the following table:

Disease	Incidence in "Hypomotility" Series	
	Carman and Miller	Levy and Kantor
Lesions of the appendix....	1.2	1.0
Lesions of the gallbladder...	5.0	3.7
Gastroduodenal ulcer	57.0	50.0 (estimated)
Gastric cancer	36.0	7.5
"Nonsurgical" conditions...	...	36.4

In the two cases of delayed emptying following gastro-enterostomy, the indications for the operation were by no means clear in the first place.

The two cases of extragastric obstruction were, respectively, an extensive retroperitoneal sarcoma in a negro, and a very large liver and spleen of undetermined etiology in a boy of 7.

Lesions of the intestine are supposed to cause gastric delay reflexly, perhaps through pylorospasm. Our series includes, in addition to appendicitis, cases of adhesions, carcinoma of the colon, mucous colitis[10] and severe constipation. In all, lesions of the intestine were present in over 5 per cent. of our cases of delayed gastric emptying.

The association of the phobias (apprehension neurosis) with digestive disturbances is no new observation. Oppenheim[11] mentions an interesting case of astrophobia which presented the symptoms of weakness, nausea, and vomiting. The patient's 8-year-old son was not afraid of storms, but showed the same digestive disturbances as his mother. Both of our patients were afraid of crowds. One of the cases was associated with autointoxication. We have records of seven other cases with digestive symptoms but without delayed gastric emptying. A possible explanation of the "hypomotility" in this condition may lie in the fact that the emotional state in these patients is in reality one of chronic fear, analagous to the acute fear induced in the animals observed by Cannon.[12] Here there was noted complete absence of peristalsis. This seemed to be the case in one of our patients; in the other the peristalsis was hyperactive.

9. Carman, R. D., and Miller, A.: The Roentgenologic Determination of Gastric Motility, THE ARCHIVES INT. MED., 1915, xvi, 406.

10. Herschell (Indigestion. London, H. J. Glaisher, p. 104) makes much of the association of mucous colitis with gastric myasthenia. The bowel symptoms, which would naturally follow from gastric "hypomotility" from any cause, are the result of the continuous discharge into the intestine of improperly digested material.

11. Oppenheim, H.: Lehrbuch der Nervenkrankheiten. Berlin, 1913, p. 1521.

12. Cannon, W. B.: The Mechanical Factors of Digestion. New York, Longmans, Green & Co., 1911, p. 217; Bodily Changes in Pain, Hunger, Fear, and Rage. New York, D. Appleton & Co., 1915, p. 16.

TABLE 2.—One Hundred Forty-One Cases of Delayed Gastric Emptying Studied Roentgenologically ("Roentgen-Ray Series")

No.	Sex	Age, Years	Residue		Fasting Contents, c.c.	Ptosis, cm.	Atony	Acidity	Water-Trap	Clinical Diagnosis	Probable Cause of Delay
			Tube	Roentgen-Ray							
1	M.	35	8½ h. D. 120	6 h. sm.	120	9	0	Hyper.	—	Duodenal ulcer	Spasm
*2	M.	60	6 h. D. 240	0	30	12.5	+	Hyper.	—	Duodenal ulcer	Spasm; atony
3	M.	46	4 h. B. +	6 h. +	—	9	++	Hyper.	—	Ptosis and atony	Atony; spasm
4	M.	23	6 h. L. +	6 h. sm.	Normal	10.5	Sl.	Hyper.	+	?	Spasm
*5	M.	37	6 h. D. 180	0	Normal	7.5	+	Normal	0	Duodenal ulcer	Atony
6	M.	50	7 h. L. 370	6 h. sm.	70	11.5	0	Hyper.	0	Ulcer	Spasm
7	M.	67	6 h. D. +	6 h. +	180	+	+	Hyper.	—	Duodenal ulcer	Spasm
8	F. M.	37	—	6 h. sm.	Normal	7	0	Normal	+	Intestinal adhesions	Spasm
*9	F. M.	45	6 h. D. 300	0	Normal	+	+	Hyper.	—	Ulcer; chronic nephr.	Spasm
*10	M.	30	6 h. D. 300	0	Normal	3.5	0	Hyper.	—	?	Spasm
11	M.	30	6 h. B. 150	6 h. sm.	—	+	+	Hyper.	—	Ptosis and atony	Atony; spasm
12	M.	54	6 h. L. 500	6 h. sm.	—	+	+	Hyper.	—	Ulcer	Atony; spasm
*13	F. M.	39	—	6 h. lge.	Normal	+	+	Hyper.	0	Ulcer; chronic endocarditis.	Spasm
			7 h. D. 10	0	(Three years later: chronic gastritis and anacidity)						
*14	M.	22	6 h. B. +	0	—	10.5	+	Hypo.	0	Ulcer	Atony
15	F. S.	17	6 h. D. ++	6 h. lge.	Normal	9.5	Sl.	Normal	—	Ulcer	Obstruction
16	M.	43	6 h. D. 300	6 h. lge.	50	3.5	Sl.	Hyper.	—	Duodenal ulcer	Spasm
17	M.	42	—	6 h. lge.	Hypers.	7.5	Sl.	Hyper.	0	Duodenal ulcer	Spasm; obstr.?
18	M.	56	6 h. D. +	24 h. +	—	13	++	Hyper.	—	Ulcer	Obstruction
19	F. M.	61	—	6 h. lge.	—	8	++	Hypo.	—	Carcinoma	Obstruction
*20	F. S.	27	6 h. D. mod.	0	Normal	6.5	0	Hyper.	—	?	Spasm
			6 h. D. lge.	6 h. sm.	(After gastro-enterostomy. No obstruction found at operation)						
*21	M.	42	6 h. D. 60	0	30	4.5	0	Hyper.	0	Duodenal ulcer	Spasm

22	F. M.	61	—	6 h. sl.	30	9	+	Hyper.	—	Autointoxication	Spasm
*23	M.	25	6 h. D. lge.	0	Normal	3.5	0	Hyper.	—	?	Spasm
24	F. M.	53	6 h. L. + 6 h. L. 0	24 h. sm.	500 F.	8.5	0	Hyper.	—	Ulcer	Obstruction
			(After gastro-enterostomy; cured)								
25	M.	24	6 h. D. +	6 h. sm.	Normal	11	Sl.	Hyper.	—	?	Spasm
*26	M.	30	6 h. B. 300	0	—	5.5	Sl.	Hyper.	0	Duodenal ulcer	Spasm
27	M.	41	12 h. L. 30	6 h. lge.	60 F.	9	Sl.	Hypo.	—	Gastric ulcer	Obstruction
28	M.	64	6 h. L. 1,000	24 h. lge.	2,000 F.	9	+	—	—	Ulcer	Obstruction
29	M.	49	—	6 h. lge.	1,000 F.	5	0	Hypo.	—	Carcinoma	Obstruction
30	M.	40	—	6 h. sm.	Normal	5.5	0	Hyper.	—	?	Spasm
*31	F. M.	45	— 7 h. D. 50	0 6 h. sm.	Normal (Two years later)	10	0	Hyper.	—	Chronic nephritis	Spasm
32	M.	41	—	6 h. mod.	Normal	0	0	Hyper.	—	?	Spasm
33	M.	28	6 h. D. sm.	6 h. sm.	30	0	0	Hyper.	—	Duodenal ulcer	Spasm
34	M.	12	6 h. D. 1,000	6 h. sm.	Food	0	0	Hyper.	—	After gastro-enterostomy	Spasm; vicious circle?
35	F. M.	59	—	6 h. lge.	500 F.	0	0	—	—	Carcinoma	Obstruction
36	M.	38	—	6 h. sm.	Normal	0	0	Hyper.	—	Duodenal ulcer	Spasm
37	F. M.	60	—	6 h. lge.	Normal	9	+	Normal	—	Cholelith.	Extra gastric obstruction
*38	M.	40	—	0	—	0	0	Hyper.	—	Duodenal ulcer	Spasm
39	F. M.	29	—	6 h. mod.	Normal	9.5	Sl.	Hyper.	0	Migraine; chronic nephr.	Spasm
*40	M.	59	6 h. D. +	0	Normal	+	+	Hyper.	—	Duodenal ulcer	Atony; spasm
41	F. S.	24	6 h. D. 500	6 h. sm.	30	13.5	+	Hyper.	—	After gastro-enterostomy	Obstruction?
*42	M.	31	6 h. D. 300	0	Normal	12	+	Hyper.	—	Epilepsy	Atony
*43	F. S.	23	6 h. B. lge. 8 h. D. 0	0	Normal	12	+	Hyper.	0	Atony	Atony
			(After gastro-enterostomy and gastropexy)								
*44	M.	40	5 h. B. lge.	0	Hypers.	4.5	0	Hyper.	+	Ulcer	Spasm
45	F. M.	50	—	6 h. mod.	Normal	3.5	0	Normal	0	?	?
46	M.	52	—	6 h. sm.	Normal	4.5	0	Hyper.	+	Ulcer; syst. tbc.	Spasm
47	M.	50	—	48 h. +	500 F.	12	+	Hyper.	—	Ulcer	Obstruction

TABLE 2.—One Hundred Forty-One Cases of Delayed Gastric Emptying Studied Roentgenologically ("Roentgen-Ray Series")—(*Continued*)

No.	Sex	Age, Years	Residue		Fasting Contents, c.c.	Ptosis, cm.	Atony	Acidity	Water-Trap	Clinical Diagnosis	Probable Cause of Delay
			Tube	Roentgen-Ray							
48	F. M.	57	7 h. D. lge.	6 h. mod.	Normal	11	+	Hypo.	—	Ptosis and atony	Atony
*49	F. S.	28	7 h. D. lge.	0	Normal	5.5	0	Hyper.	0	Migraine	Spasm
50	M.	42	—	6 h. sm.	22	8	Sl.	Hyper.	0	Duodenal ulcer	Spasm
51	F. S.	26	—	6 h. +	Normal	10	+	Hyper.	—	Autointoxication	Spasm; atony
*52	M.	42	7 h. D. lge.	0	Normal	11.5	Sl.	Hypo.	+	Duodenal ulcer	Atony; water-trap?
53	M.	35	—	4 h. lge.	—	0	0	—	—	Retroperit. lymphosarc.	Obstruction
			(No Roentgen-ray examinations after four hours; practically entire meal left then)								
54	F. M.	81	—	6½ h. sm.	Hypers.	11.5	+	Normal	—	Ptosis and atony	Atony
55	F. M.	33	6 h. D. mod.	6 h. sm.	Hypers.	3	Sl.	Hypo.	—	Autointoxication; migraine	Spasm; atony?
*56	M.	22	6 h. D. mod.	0	—	0	0	Normal	—	?	?
*57	M.	37	6 h. D. mod.	0	Normal	10.5	+	Hyper.	+	Duodenal ulcer	Spasm; atony
58	M.	65	—	24 h. +	500 F.	4	0	Hypo.	0	Carcinoma	Obstruction
59	F. M.	62	—	6 h. lge.	Normal	13	Sl.	Hypo.	+	Tuberculosis	Atony; water-trap?
60	M.	13	—	6½ h. lge.	Food	0	0	Normal	—	Adhesions, intestinal	Spasm
*61	M.	32	8 h. L. mod.	0	—	14	+	Hyper.	—	Gastric ulcer	Spasm
62	M.	38	—	6 h. +	Normal	0	0	Normal	0	Duodenal ulcer	Spasm
*63	F. M.	45	6 h. D. +	0	Normal	8.5	+	Hypo.	+	Cholelith	Spasm
64	F. M.	50	—	6 h. lge.	Normal	6	Sl.	Normal	0	Autointoxication	Spasm
65	F. S.	20	—	9 h. +	Normal	10.5	0	Normal	0	Tuberculosis	Spasm
66	M.	45	—	6 h. lge.	—	6.5	0	—	—	Chronic appendicitis	Spasm
67	M.	37	6 h. D. 300 7 h. D. 200	7 h. + 0	Food (After gastro-enterostomy moderate improvement)	10	+	Hyper.	+	Duodenal ulcer	Obstruction

68	M.	42	5 h. B. 100 8 h. L. 90	6 h. lge.	Food	13	0	Hyper.	+	Duodenal ulcer	Obstr.; spasm
					(After gastro-enterostomy moderate improvement)						
69	M.	62	6 h. D. +	6 h. lge.	—	12.5	+	Hyper.	—	Duodenal ulcer	Spasm; atony
*70	M.	31	6 h. D. 120	0	Normal	8	0	Hyper.	0	Duodenal ulcer	Spasm
*71	F. S.	29	7½ h. D. sm.	0	Normal	10	0	Normal	0	Autointoxication	Spasm
72	F. S.	28	7 h. D. +	6 h. mod.	Normal	0	0	Normal	0	Gastric ulcer	Spasm
73	M.	40	6 h. L. lge.	6 h. lge.	Food	7.5	+	Hyper.	—	Ulcer	Obstr.; spasm; atony
74	F. M.	26	—	6 h. lge.	Normal	5.5	+	Normal	0	?	Atony
75	M.	33	7 h. D. lge.	24 h. +	145 F. Sl.	8	++	Hyper.	—	Duodenal ulcer	Spasm
76	M.	58	6 h. D. lge.	24 h. +	Food	14	++	Hyper.	—	Ulcer	Obstruction
77	F. S.	23	6 h. D. lge.	6½ h. lge.	Normal	0	0	Normal	0	?	?
78	M.	53	6 h. D. +	6 h. sm.	Hypers.	9.5	++	Normal	—	Ulcer	Spasm
79	M.	41	6 h. D. lge. 6 h. D. lge.	5½ h. lge. —	Food	0	0	Normal	0	Duodenal ulcer	Obstruction
					(After gastro-enterostomy)						
80	M.	46	6 h. D. +	6 h. lge.	Normal	0	0	Hyper.	0	Duodenal ulcer	Spasm
81	F. M.	41	6 h. D. 300	6 h. sm.	Normal	10.5	+	Hyper.	0	?	Spasm; atony
82	F. S.	51	6 h. D. lge.	6 h. lge.	Normal	9.?	0	Hypo.	0	Autointoxication	Spasm?
83	M.	41	—	6 h. sm.	Hypers.	4.5	Sl.	Hypo.	+	Autointoxication; apprehension	Spasm; atony
*84	M.	46	6 h. D. lge.	0	Normal	4	0	Normal	0	Migraine	Spasm
*85	M.	31	6 h. D. 300 7 h. D. +	0	—	10	Sl.	Hyper.	0	Duodenal ulcer	Spasm
86	M.	46	6 h. D. 200	6 h. sm.	60	7.5	+	Hyper.	—	Duodenal ulcer	Spasm
*87	M.	45	6 h. D. lge.	0	Normal	5.5	0	Hypo.	—	Tuberculosis, pulmonary	Spasm?
88	F. M.	43	—	6 h. mod.	Normal	0	0	Hypo.	0	Nephrolith?	Spasm?
89	F. M.	46	—	6 h. mod.	Normal	10.5	+	Hypo.	0	Chronic appendicitis; autointoxication	Atony; spasm?
90	F. S.	26	6 h. D. 240	6 h. lge.	Normal	10	+	Normal	—	Bone tuberculosis	Atony
91	F. M.	52	6 h. B. 90	7 h. mod.	Normal	7.5	0	Hyper.	0	Duodenal ulcer; autointoxication	Spasm

TABLE 2.—One Hundred Forty-One Cases of Delayed Gastric Emptying Studied Roentgenologically ("Roentgen-Ray Series")—(*Continued*)

No.	Sex	Age, Years	Residue		Fasting Contents, c.c.	Ptosis, cm.	Atony	Acidity	Water-Trap	Clinical Diagnosis	Probable Cause of Delay
			Tube	Roentgen-Ray							
92	M.	7	—	6 h. sm.	—	3.5	0	—	—	Enlarged liver and spleen...	Extra gastric obstruction
93	F. M.	34	6 h. D. lge.	7 h. sm.	40	10.5	0	Normal	—	Intestinal adhesions.....	Spasm
94	F. M.	31	7 h. D. 100	6 h. lge.	—	11	Sl.	Hyper.	—	Ptosis and atony.....	Spasm; atony
95	M.	68	—	24 h. lge.	2,000 F.	7.5	++	Hypo.	—	Ulcer.....	Obstruction
*96	M.	44	7 h. D. 170	0	Normal	9	0	Hyper.	0	Mucous colitis.....	Spasm
97	F. M.	56	6 h. L. 200	6 h. sm.	Normal	10.5	+	Normal	0	Ptosis and atony.....	Atony
98	F. M.	33	7 h. D. mod.	6 h. sm.	Normal	6	0	Hypo.	—	Constipation.....	Spasm?
99	M	33	7 h. L. 100	6 h. lge.	140	10	0	Hyper.	0	Duodenal ulcer.....	Spasm
100	F. S.	21	—	6 h. mod.	54	7.5	0	Hyper.	0	?	Spasm
101	F. M.	48	—	6½ h. mod.	Normal	16	+	Hyper.	0	Chronic appendicitis?.....	Spasm
102	M.	45	—	6 h. sm.	Normal	5.5	0	Hyper.	0	?	Spasm
103	F. M.	52	6½ h. D. 200	6 h. sm.	Normal	3	0	Hypo.	0	(Chronic endocarditis; achylia gastrica)	?
104	F. M.	28	—	6¼ h. mod.	Normal	10	0	Normal	0	Appr. neur.	Spasm
105	F. M.	32	6¾ h. D. 80	6 h. mod.	Normal	11.5	0	Normal	0	(Chronic endocarditis).....	?
106	M.	65	—	19½ h. lge.	300 F.	4	Sl.	Hypo.	—	Ulcer.....	Obstruction
107	F. M.	47	7 h. D. 150	6 h. sm.	Normal	0	0	Hyper.	0	?	Spasm
108	M.	34	7 h. D. 150	6½ h. sm.	—	0	0	Hyper.	0	Duodenal ulcer.....	Spasm
*109	M.	52	7 h. D. 250	0	45	0	0	Hyper.	0	(Arteriosclerosis).....	Spasm
110	M.	24	8 h. L. 145	6 h. lge.	165	7	0	Hyper.	—	Ulcer.....	Spasm
111	F. M.	37	6 h. D. 50	6 h. sm.	40	9.5	+	Achylia	0	Ptosis and atony (lactating)	Atony
112	M.	65	6½ h. D. 150	9 h. +	Normal	3.5	0	Normal	+	Ulcer.....	Spasm
113	F. M.	46	6½ h. L. 650	24 h. lge.	400 F.	9	Sl.	Hypo.	0	Ulcer.....	Obstruction

114	M.	9	—	6 h. sm.	Normal	0	0	Hypo.	0	(Nephritis).....	Spasm
115	F. S.	27	—	6¼ h. lge.	—	0	0	—	+	Adhesions.....	Spasm? Water trap?
116	F. M.	35	7 h. D. 100	8½ h. sm.	Normal	9.5	+	Hypo.	—	Ptosis and atony.....	Atony
117	F. M.	30	7 h. L. 50	6 h. lge.	Normal	13	+	Normal	—	Ptosis and atony.....	Atony
118	M	50	6 h. B. 80	6 h. lge.	270 F.	5.5	Sl.	Hyper.	—	Ulcer.....	Obstr.; spasm
119	F. M.	27	7 h. D. 40	6 h. mod.	72	13.5	+	Normal	0	Ptosis.....	Atony
120	F. M.	54	6 h. B. 100	24 h. +	210	11	Sl.	Hypo.	0	Cholelith.	Spasm; obstr.
121	F. M.	23	7 h. D. 70	9½ h. mod.	Normal	10.5	0	Normal	—	Autointoxication.....	Spasm
122	F. M.	53	—	24 h. lge.	400 F.	13	Sl.	Hyper.	—	Ulcer.....	Obstruction
123	F. S.	18	7¼ h. D. 50	6¼ h. mod.	40	4	0	Normal	0	?	?
124	M.	34	7 h. B. 1,500	24 h. lge.	900 F.	10.5	+	Normal	—	Ulcer.....	Obstruction
125	M.	62	7¼ h. D. 15	6 h. mod.	Normal	16	+	Normal	+	Ptosis and atony.....	Atony
126	F. M.	55	—	7 h. mod.	100	14	Sl.	Hyper.	—	Cholelith.	Spasm; obstr.
127	M.	71	6 h. B. 300	6 h. sm.	Normal	3	0	Hyper.	—	Ulcer.....	Spasm; obstr.
*128	M.	53	5¼ h. B. 150	0	—	13	0	Hyper.	0	Duodenal ulcer.....	Spasm
129	F. M.	64	7 h. B. 80	6 h. mod.	Normal	18	+	Hypo.	—	Ptosis and atony.....	Atony
130	F. S.	19	7 h. D. sl.	6 h. sm.	Normal	7.5	0	Normal	+	?	Water-trap?
131	F. S.	24	7¼ h. D. 150	6¼ h. mod.	Normal	13.5	+	Hypo.	0	Ptosis and atony.....	Atony
132	F. M.	55	—	6¼ h. mod.	Normal	12	0	Achylia	0	Cholelith.	Spasm
133	F. S.	26	—	6 h. sm.	Normal	10	0	Hyper.	0	Adhesions?.....	Spasm
134	M.	40	5½ h. B. 80	6 h. mod.	—	7.5	0	Hyper.	+	Duodenal ulcer.....	Spasm
135	M	40	7 h. D. lge.	22 h. mod.	Tr. F.	7.5	+	Normal	—	Ulcer.....	Obstr.; atony
136	F. S.	28	7 h. B. 60	6 h. sl.	Normal	12	0	Hyper.	+	Autointoxication; chronic appendicitis?	Spasm
137	F. M.	60	—	6 h. lge.	1,300 F.	19.5	0	—	—	Ulcer.....	Obstruction
138	M.	67	7¾ h. L. 300	6 h. sm.	—	0	0	Hypo.	0	Cancer of colon?.....	Spasm
139	F. M.	61	5½ h. B. 40	6 h. mod.	Normal	9	+	Hyper.	+	Ulcer.....	Spasm
140	M.	38	7 h. D. 80	6 h. mod.	95	9	0	Hyper.	+	Ulcer.....	Spasm
141	M.	27	—	6 h. sm.	Normal	15.5	0	Normal	0	?	Spasm

EXPLANATION OF ABBREVIATIONS IN TABLE 2

B=Motor breakfast (e. g., 7 h. B. lge.=large residue seven hours after motor breakfast).

D=Motor dinner.

F=Food.

F. M.=Female, married.

F. S.=Female, single.

h=Hours.

L=Motor lunch.

M=Male.

An asterisk (*) means tube residue but no Roentgen-ray residue.

A dash (—) means absence of data.

Wherever possible, exact figures are given. Thus, 500 F. means 500 c.c contents with food. Otherwise, descriptive terms are used, as slight (sl.), moderate (mod.), large (lge.). A zero means the absence of a condition — in the "X-Ray-residue" column it means no six hour residue — a plus sign the presence of the condition; two plus signs its presence in a marked degree.

In the "Fasting Contents" column an amount under 50 c.c is considered normal, over 50 c.c. is hypersecretion; exact figures being given where available. In the "Acidity" column "hyper" means hyperchlorhydria, hyperacidity, or both. "Hypo" means hypochlorhydria, hypoacidity, or anacidity. The normal limits implied are: Free HCl 20 to 40 degrees; total acidity 40 to 60 degrees.

Our clinical findings may be summarized by saying that 74 per cent. of the cases of delayed gastric emptying were due to causes residing within the digestive tract, whereas 25.1 per cent. were due to causes outside the tract. The stomach itself was the seat of the trouble in 65.1 per cent. of the cases.

VI. CONCLUSIONS

1. A rational test for gastric emptying should be based on the patient's ability to get rid of a meal of ordinary bulk and complexity in the usual period, namely, in about seven hours. The "fasting contents" tube test, and the Roentgen-ray test, as commonly employed, are not sufficiently delicate for many clinical purposes.

2. Approximately one in every eight or nine patients with digestive complaints suffers from delayed gastric emptying.

3. The chief factors to be considered in working out a case of delayed gastric emptying are: (a) shape of stomach, (b) character of motor meal, (c) tone of stomach wall, (d) acidity of gastric juice, (e) nervous influences, (f) mechanical obstruction.

4. The immediate causes of delayed gastric emptying and their relative frequency are: (a) spasm of pylorus in 58.8 per cent. of the cases, (b) obstruction at pylorus in 22.2 per cent., (c) atony of stomach in 17 per cent.

5. The conditions most commonly predisposing to or associated with delayed gastric emptying are: (a) ulcer in about 50 per cent. of the

cases, (b and c) autointoxication and gastric cancer, each 7.5 per cent., (d) ptosis with atony, 7 per cent., (e) cholelithiasis, 3.7 per cent.

6. The causes of delayed gastric emptying are located in the stomach in 75.3 per cent. of the cases, elsewhere in the digestive tract in 10.3 per cent., and outside the digestive tract in 13.4 per cent.

717 East Genesee Street.

FURTHER STUDIES OF BIOLOGICAL METHODS FOR THE DIAGNOSIS OF TUBERCULOSIS *

J. BRONFENBRENNER, PH.D., MORRIS H. KAHN, M.D., J. ROCKMAN, M.D., AND MAX KAHN, M.D., PH.D.
PITTSBURGH

It is a well accepted truth among laboratory workers that biological methods of diagnosis are only relatively specific. This limited usefulness of biological tests is true not only for such as the Abderhalden reaction, for instance, which is still in process of development, but equally true when applied to such widespread tests as Widal and Wassermann reactions.

Theoretically, inasmuch as these reactions depend on the specific biological processes taking place under the conditions of the disease, the biological methods of diagnosis should be strictly specific. The ideal method will be the one by which positive results are obtained in 100 per cent. of cases presenting symptoms of the disease, at the same time giving uniformly negative results on examination of individuals not affected by the disease.

The accumulated experience of a number of investigators through a period of years has shown, however, that practically every method is subject to limitations in its specificity, as well as in its sensitiveness, even if performed by experts.[1]

It is generally accepted now, for instance, that a negative Wassermann reaction is only of relative value, and many experienced clinicians are inclined to suspect the positive Wassermann test also when not corroborated by the clinical picture of the case, or by previous history. The agglutinins and precipitins are generally accepted to be mainly group reactions, and only if quantitatively considered may they be of diagnostic value.

The difficulties in the diagnosis of tuberculosis by biological methods seem to be especially great. Even when specific in character, some methods are of but little diagnostic significance on account of extreme sensitiveness, as the von Pirquet test, for example.

---

* Submitted for publication Jan. 20, 1916.

* From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, and the Tuberculosis Clinic of the Department of Health, New York City.

* Read before the Society for Serology and Hematology, New York, Dec. 3, 1915.

1. In addition, the complexity of methods opens a wide field of errors in diagnosis, due to faulty technic.

Many attempts have been made to apply to the diagnosis of tuberculosis methods on which the diagnosis of other infectious diseases are based, as, for instance, the agglutinin, the precipitin, the meiostagmin reaction, and lately, the biochemical test of Abderhalden; but the results obtained were of scientific interest only. The difficulties of applying methods which work satisfactorily in the diagnosis of other diseases, to tuberculosis is especially apparent in case of the complement deviation test. Numerous attempts to work out this method in tuberculosis have been made by many investigators, but on account of the peculiar properties of the organism, it has not been possible to get a preparation of antigen which could be used for this test successfully. Professor Besredka,[2] who succeeded in cultivating tubercle bacilli on an entirely new medium, was induced by the fact that on this medium the organism showed some hitherto unknown properties, to try also the antigenic properties of his new cultures for the complement deviation test. Successful results obtained by Besredka and Manoukhine[3] in their preliminary experiments, were fully confirmed in this laboratory.[4] We found that, although not fulfilling all the requirements of an ideal diagnostic test, the complement deviation with Besredka's tuberculin seems to be very useful. In fact, it was positive in 93 per cent. of cases of clinical tuberculosis. At the same time the occurrence of the reaction in cases in which tuberculosis could not be detected clinically, was limited to less than 10 per cent. out of a total of several hundred.[5] More recently many investigators have confirmed the diagnostic value of the complement deviation test with the tuberculin of Besredka, and in the hands of some of them,[6] the occurrence of the reaction among patients presenting clinical symptoms of the disease is even as high as 95 per cent. In controlling the test by a Wassermann reaction,[7] we have been surprised from the beginning, to find that a comparatively large number of serums, reacting with the tuberculin of Besredka, showed a positive Wassermann test[8] also.

2. Besredka: Compt. rend. Acad. d. sc., 1914, clvi, 1633.

3. Besredka and Manoukhine: Compt. rend. Soc. de biol., 1914, lxxvi, 180.

4. Bronfenbrenner: Proc. Soc. Exper. Biol. and Med., 1914, xi, 92.

5. Bronfenbrenner: Ztschr. f. Immünitatsf., Orig., 1914, xxiii, 221.

6. Inman: Lancet, London, March, 1914, p. 4734; also Compt. rend. Soc. de biol., 1914, p. 251. Renaut: Compt. rend. Soc. de biol., 1914, p. 865. Debains et Jupille: Compt. rend. Soc. de biol., 1914, p. 199.

7. As the tuberculin contains lipins derived from the medium on which the tubercle bacilli are grown, and thus there was a possibility that certain nontuberculous serums having lipotropic properties, as for instance syphilitic serums, might fix the complement with this antigen, this control was deemed necessary.

8. Bronfenbrenner: Proc. Soc. Exper. Biol. and Med., February, 1914, p. 92.

INTERFERENCE OF LIPINS WITH ANTIGENIC PROPERTIES OF TUBERCULIN

The first explanation of the coincidence of the positive tuberculosis and Wassermann reactions naturally was that the serum which had a high lipotropic coefficient fixed with tuberculous antigen also on account of the lipins it contains.

We[9] studied this possibility in a number of cases and found that whenever the complement deviation with Besredka antigen is present in serums with high lipotropic properties, it depends on the presence of a separate tuberculous antibody possessing its own index, different from the lipotropic index of the same serum. We found, moreover, that the lipins which can be extracted from the tuberculin with ether, chloroform, benzin or petroleum ether, are not sufficient, in the quantity in which they are present in the tuberculin, to cause a fixation of the complement in the presence of lipotropic serums.

In order to avoid even the possibility of such nonspecific reaction, however, we repeated our earlier suggestion to deliponize the tuberculin.[9]

In a large series of experiments performed in this laboratory by Dr. J. Rockman, in which the tuberculin of Besredka was deprived of lipins by means of extraction by ether, it was found that the antigenic properties of the tuberculin were not injured thereby.[10] In later experiments instead of extracting the lipins from the antigen of Besredka, we succeeded in isolating the active principle of the tuberculin by means of precipitation with ten volumes of absolute alcohol. Another efficient method was the precipitation of protein from the tuberculin by adding two drops of glacial acetic acid to 10 c.c. of Besredka tuberculin, which produced a heavy precipitate. The precipitate, when centrifuged, suspended in 10 c.c. of physiological salt solution and neutralized to phenolphthalein, proved to be an excellent antigen, practically free from any lipin except the small amount adherent to the precipitate.[11]

Although, as we previously said, in our early work with the first sample of the tuberculin of Besredka our conclusions were that the amount of lipins present was not sufficient to cause nonspecific fixation of complement in the presence of highly lipotropic serums, the importance of freeing the tuberculin of all its lipins became especially evident in later experiments. We noticed that different samples of tuberculin received by us at different times from Professor Besredka's laboratory, differed in their antigenic properties. The most striking

9. Bronfenbrenner: THE ARCHIVES INT. MED., 1914, xiv, 786.
10. Similar findings were reported since, also by Renaut: Compt. rend. Soc. de biol., 1914, lxxvi, 865.
11. Bronfenbrenner and Rockman: Biochem. Bull., 1914, iii, 381.

feature of the differences noticed in a large series of cases was the apparent power of one sample especially (No. 4) to fix complement in presence of nontuberculous lipotropic serums. The study of this phenomenon revealed that the cause for such irregularity in the behavior of the sample of Tuberculin 4 was due to the comparatively high lipin content.

The actual quantitative estimate of lipins in different samples of the tuberculin of Besredka was made as follows: 10 c.c. of each of the different samples were precipitated with 2 drops of glacial acetic acid, thus removing the coagulable protein from the tuberculin. The coagula were repeatedly extracted with alcohol-ether mixture and the extracts thus obtained were evaporated to dryness. The resulting residues of lipins were taken up with 0.2 c.c. of methyl alcohol and each sample was diluted to the original volume of tuberculin (10 c.c.) by the addition of the respective supernatant fluid resulting from the precipitation by acetic acid of coagulable protein from each sample of tuberculin.

These emulsions of lipins were then used as if they were syphilitic antigen in parallel series with a preparation of lipins originating from beef heart, which we are using for the routine diagnosis of syphilis.[12] All the emulsions were tested in varying quantities against a constant amount of complement, amboceptor and known syphilitic serum.

TABLE 1.—Comparative Data on Lipin Fractions from Besredka Tuberculin as Syphilitic Antigens

	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.	0.005 c.c.	0.003 c.c.
Syphilitic antigen	+	+	+	+	+	+	±	±	−
Lipins from Tuberculin IV...	+	+	±						
Lipins from Tuberculin III..	−	−	−						
Lipins from Tuberculin II...	−	−	−						

It was found that, when concentrated many times, the emulsions of lipins from Tuberculin II, and especially III, were also able to fix the complement with syphilitic serums, but, *in the amount in which they were present in the tuberculin*, they were not able to cause the nonspecific fixation (Table 1). The sample of Tuberculin IV, however, as it appears from Table 1, contained originally an amount of lipins which in the dose of 0.05 c.c. of the original concentration in the tuberculin already caused fixation of complement with leutic serums. If such tuberculin is used in its original form in the complement deviation test with serums possessing a high lipotropic index, it is evident that a certain number of nonspecific fixations may occur, as can be seen from Table 2.

12. Noguchi and Bronfenbrenner: Jour. Exper. Med., 1911, xiii, 43.

TABLE 2.—Comparative Frequency of Fixation Occurring with Different Samples of Tuberculin (Nonpurified) *

Twenty-Five Cases of Each Group		Tuberculin II	Tuberculin III	Tuberculin IV	Pure Lipoid (Wassermann)
No Diagnosis of syphilis....	Tuberculosis suspected.....	24	22	6	0
	Tuberculosis not suspected	1	1	0	0
Diagnosis of syphilis.....	Tuberculosis suspected.....	23	22	14	25
	Tuberculosis not suspected	4	5	11	25

* This table was composed by selecting from the records of all cases examined twenty-five for each of the four groups, according to clinical diagnosis with the corresponding serological findings in each case.

As Table 2 shows, the sample of Tuberculin IV gives a very high number of fixations in cases of serums with high lipotropic power, irrespective of tuberculous infection. Whereas with the Tuberculins II and III, the occurrence of positive reactions was equal to 16 to 20 per cent. in cases in which the Wassermann test was also positive, but tuberculosis not suspected prior to the serological examination;[12] the Tuberculin IV gave 44 per cent. of positive reactions in the same cases.

The higher occurrence of fixations with the Sample IV could be attributed to two causes—either this sample was more efficient in detecting tuberculous antibody in the serum, or, on account of its high content of lipin the sample of Antigen IV caused a certain number of fixations of purely lipotropic character and not specific as far as diagnosis of tuberculosis is concerned.

Comparing the frequency of occurrence of the fixation with different tuberculins in the cases of serums with positive and negative Wassermann reactions, respectively, we notice that Tuberculin IV is less efficient in fixing the complement with nonsyphilitic serums than with serums giving a positive Wassermann. We see that in cases diagnosed as tuberculosis with positive as well as with negative Wassermann, its efficiency is much lower than that of Tuberculins II and III. These findings speak definitely against the possibility of the higher specific efficiency of Tuberculin IV being the reason for the greater number of fixations referred to above. It is evident, therefore, that the high percentage of fixations obtained with this sample of tuberculin in cases with positive Wassermann, is due to a certain number of lipotropic reactions.

Although in a number of cases of syphilis we have been able to demonstrate the presence of the two independent antibodies,[9] whenever both the Wassermann and tuberculosis fixation tests were positive, *it is evident that such may not be the case if the antigen used contains an*

13. This is about the same percentage as we observed in prior series.

excess of lipins. It is therefore imperative to free such a sample of tuberculin of its lipins before using it for the complement deviation work. As a method of doing it, we have found[14] the precipitation with acetic acid to be the simplest.

COMPARATIVE ANTIGENIC VALUE OF DIFFERENT SAMPLES OF TUBERCULIN OF BESREDKA

As Table 2 shows, in the twenty-five cases of tuberculosis in which the Wassermann reaction was negative, and there was no chance of complication due to the lipotropic properties of the serums, the occurrence of positive reactions with Tuberculins II, III and IV was 24, 22 and 6, or 96, 88 and 24 per cent., respectively.

TABLE 3.—DETERMINATION OF ANTIGENIC UNIT OF THE PROTEIN FRACTION OF DIFFERENT SAMPLES OF TUBERCULIN OF BESREDKA *

		Amount of Tuberculin Used (in Cubic Centimeters)											
		0.3	0.2	0.15	0.1	0.07	0.05	0.03	0.02	0.015	0.01	0.007	0.005	0.003
Pulmonary tuberculosis A	Tuberculin II	+	+	+	+	+	+	+	+	+	+	+	±	±
	Tuberculin III	+	+	+	+	+	+	+	+	+	±	−		
	Tuberculin IV	+	+	+	+	+	+	+	±	−				
Pulmonary tuberculosis B	Tuberculin II	+	+	+	+	+	+	+	+	+	±	−		
	Tuberculin III	+	+	+	+	+	+	+	±	−				
	Tuberculin IV	+	+	+	+	+	+	±	−					
No tuberculosis C	Tuberculin II	+	+	±	−									
	Tuberculin III	+	+	±	−									
	Tuberculin IV	±	−	−										

* The two serums selected for Table 3 were derived, respectively, from cases of first and third stages of pulmonary tuberculosis. We purposely selected these two cases out of the number of serums used for titration of antigens in order to show that the smallest amount of a given antigen sufficient to cause the fixation of complement in presence of one specific serum may not be sufficient when another serum is used. The reason for this difference is the inequality in the amount of antibody in different specific serums. It is therefore imperative always to use in a routine test not the smallest amount of antigen which was found to be sufficient to fix the complement in presence of a certain serum (unit of antigen), but several times that amount, so as to be sure to obtain fixations also in cases in which the amount of specific antibody is smaller. In the cases above, the respective units of Antigens II, III and IV were 0.007 c.c., 0.015 c.c., and 0.03 c.c. in case of the serum of a primary stage, and 0.015 c.c., 0.08 c.c. and 0.05 c.c. in the case of tertiary stage, which indicates that the amount of antibody present in the first case is greater than in the second.

Remembering that the percentage of occurrence of fixation in similar cases reported by us before with Tuberculin I was equal to 93.84, it is evident that in respect to specific properties different preparations of

14. We were trying to further purify the tuberculin of Besredka, but our work was interrupted by unforeseen conditions preventing us from obtaining further supplies of tuberculin from Professor Besredka, and will be resumed as soon as possible. We wish at this time to express our appreciation to Prof. W. J. Gies for his valuable suggestions as to the methods to be employed in further chemical purification of the tuberculin.

tuberculin of Besredka vary markedly. In trying to explain this variation in antigenic power in different samples of tuberculin, we naturally suspected the inequality of concentration of specific elements in respective preparations of tuberculin.

In order to avoid this source of error we decided to isolate the protein fraction of tuberculin in each case by means of precipitation, and to use the equivalent antigenic doses for the test instead of diluting each tuberculin, as before, to the original volume.

Ten cubic centimeters of each of Tuberculins II, III and IV were precipitated with two drops of glacial acetic acid. Each precipitate was separated by centrifuging, suspended in 3 c.c. of salt solution and neutralized to phenolphthalein. The resulting solutions were titrated for their antigenic values against several serums of active pulmonary tuberculosis by the regular complement deviation method. The results are shown in Table 3.

Having thus obtained the respective antigenic units of different samples of the coagulable protein fraction of tuberculins, we used in the subsequent tests equal antigenic values of each; namely, in case of Tuberculin II, 0.03 c.c.; in case of Tuberculins III and IV, 0.07 c.c. and 0.15 c.c., respectively (about 5 antigenic units).

Although in general the results became more comparable than before, especially as the lipotropic reactions in Sample IV were absent, there were still a certain number of cases of tuberculosis in which (specially when the amount of antibody in the serum was not great) the reaction was positive only with either one or two of the three samples of tuberculin used.

TABLE 4.—The Comparison of the Occurrence of Fixation with Different Samples of Purified Tuberculin

		Results Obtained								Total Occurrence of Fixations		Total No. of Cases
										Tuberculosis Per Cent.	Controls* Per Cent.	
Sample of tuberculin	II	+	+	+	+	—	—	—	—	92	8	
	III	+	+	—	—	+	—	+	—	88	8	
	IV	+	—	—	+	+	+	—	—	36	2	
Cases of diagnosed tuberculosis		18	26	2	0	0	0	0	4	..	..	50
Controls*		2	5	1	0	0	0	1	91	..	..	100

* In this number are included cases in which the infection with tuberculosis was not suspected at the time of the test.

That the differences shown in Table 4 in the frequency of the occurrence of fixation with different samples of tuberculin were not due to faults of technic was shown by the fact that the same results were obtained in repeated examinations.

Each case in which the findings obtained by different antigens did not coincide was reexamined repeatedly, and only such cases were included in the table in which repeated examinations revealed the permanent character of the discrepancy. In view of the fact that the possible differences in the amounts of specific constituents in respective samples of tuberculin were eliminated by purification and subsequent titration, the variations in antigenic efficiency recorded above can be explained only on the basis of *qualitative differences inherent in the specific constituents* of respective samples of tuberculin.

If we seek causes that account for the evident differences in apparently similar samples of Besredka tuberculin, several possible answers suggest themselves. Since the tuberculin consists of the culture medium in which tubercle bacilli have grown for a certain length of time (and there necessarily exist a number of possible variations affecting the rate of growth of different lots of cultures), different cultures are likely to contain variable amounts of the metabolic products of the organism. A further source of variation lies in the fact that in the process of preparation tuberculin is heated under pressure to kill the tubercle bacilli and some unrecorded variations in the heating process might cause partial destruction of the antigenic value of certain batches of the tuberculin.

THE STRAIN SPECIFICITY OF TUBERCULINS

As we have stated previously,[9] the complement deviation test with Besredka's tuberculin may be positive in cases in which no clinical evidence of tuberculosis could be found at the time of serological examination. In studying the question of fixation in such cases we used for control the von Pirquet test. We have shown that in all cases in which the serum test was positive the von Pirquet was also present, whereas many cases with a positive von Pirquet reaction, gave a negative serum test. In a number of cases in which the serum findings were the first to call attention to the possible presence of active tuberculosis, the subsequent histories of the cases have confirmed the early serological findings. However, in a number of cases in which this test was used there were many cases in which the subsequent study of patients was not possible and the positive serological findings in such cases remained unconfirmed by clinical data.[9]

In view of the foregoing findings, which show that in certain cases the positive findings with one sample of tuberculin may not be confirmed by examination with another sample of tuberculin of Besredka, we con-

sidered it interesting to study further the reliability of complement fixation test in clinically nontuberculous cases.

During the last twenty months we examined over 4,000 serums.[15] Among this number in 232 instances we obtained positive findings with either one or two samples of the tuberculin of Besredka, when clinically tuberculosis was not suspected at the time of the serum test. Each of the 232 serums thus selected was reexamined with the tuberculin of Besredka, and if the reaction obtained was invariably positive, each case was subsequently examined with seven different preparations of tuberculin.[16] We found that in 167 cases (or 72 per cent.) out of 232 the fixation was obtained also with at least four antigens out of seven used. Of the remaining 65 cases in 23 at least two tuberculins fixed the complement and in 12 cases CTBH was the only one confirming the results obtained with the tuberculins of Besredka. In 30 cases out of a total of 232 the fixation was obtained only with the tuberculins of Besredka.

It is thus evident that among 232 cases in which tuberculin of Besredka caused the fixation of complement, in almost 87 per cent. of the total number one or more of other tuberculins also fixed the complement. The difference in frequency of occurrence of reaction with respective antigens in case of different serums is not due to technical error. This is evident from the fact that in many instances in which the same serum happened to be examined repeatedly, the results of such examinations invariably agreed. This permanent character of discrepancies in the results obtained by using various tuberculins suggests as the only possible explanation the existence of selective specificity of the tuberculous antibody.

The specific nature of the selective fixation was especially apparent in cases in which the serum fixed complement only with the tuberculins containing human tubercle bacilli, or a mixture of human and bovine

15. Each serum coming to the laboratory for any serological examination was also examined for tuberculosis.

16. We used for this purpose the Crude Tuberculin of the Board of Health of New York (CTBH), "Old Tuberculin" Höchst (OTH), "Old Tuberculin" of Parke, Davis & Co. (OTPD), "Old Tuberculin" of Mulford containing human TB (OTMH), "Old Tuberculin" Mulford containing bovine TB (OTMB), "Bacillary Emulsion" Parke, Davis & Co. (BEPD) and our own polyvalent Bacillary Emulsion containing human as well as bovine TB (BEWPH). Each of the seven antigens was titrated against a serum from a case of active tuberculosis and their respective units were determined. In the complement deviation test 5 units of each antigen were used.

We take this opportunity to thank most sincerely Prof. W. H. Park of the New York City Board of Health, as well as Dr. E. M. Houghton of Parke, Davis & Co., and Dr. A. P. Hitchens of Mulford Company, who kindly placed at our disposal large amounts of their tuberculins.

strains, but not with the tuberculin "OTMB," for instance, containing only bovine strains. There were in our series, however, a number of cases which fixed the complement with this tuberculin, as well as with human, and in two cases we obtained the fixation only with bovine antigen, and not with human.

It is thus evident that there is in tuberculosis a certain degree of strain specificity and this contributes to the failure of certain tuberculins to answer the requirement for an antigen in the complement deviation test. The fact that different samples of the tuberculin of Besredka may differ in regard to the number of strains, and respective amounts of each, in given tuberculin preparations, would also account for the variations in antigenic values of Preparations II and III as observed above.

The amount of material at hand is not sufficient to allow of any classification of the tuberculins named, as to their comparative antigenic value, but even the results obtained already show that the antigen of Besredka may be of the greatest usefulness in the complement deviation test in cases of early nondiagnosed tuberculosis, as we have stated before,[9] especially if the number of strains composing tuberculin be further increased and the final product purified.

We have shown that out of 4,000 cases in which tuberculosis was not suspected at the time of the serum test, in 232 instances the complement deviation with Besredka's tuberculin was positive. We have shown, also, that such fixations were not due to a technical imperfection of the test, inasmuch as the fixation in these cases was obtained on repeated examinations. Moreover, we showed that in 202 out of 232 of such cases, complement fixations were obtained also by the use of other tuberculins. The question arises whether we shall consider the fixation in these cases as specific, especially in the thirty cases in which the antigen of Besredka was the only one to cause fixation.

Our previous studies[4, 9] of the subject showed that the value of this fixation is very suggestive, inasmuch as in many cases more careful subsequent examination revealed the presence of disease; and in several cases in which no clinical confirmation of serum findings could be obtained at the time, later—in the course of a year—many came to be considered tuberculous clinically. The results of the complement deviation test, however, *were not confirmed in all cases* by subsequent clinical observations.

One must not forget, however, that in the group of cases considered clinically as nontuberculous, are certainly a number of cases of latent and spontaneously cured tuberculosis. In fact, considering the results of injection of tuberculin, or the findings at necropsy, we may safely say that the majority of individuals, considered nontuberculous,

are not free from latent infection. And from this point of view it is very difficult to say with certainty whether the serum findings in such cases are correct or not.

There is only one way of controlling the serum findings in such cases with any degree of certainty, and that is by injecting tuberculin in all cases which come for serum diagnosis. But inasmuch as such procedure is not free from danger, we could not adopt it.

On the other hand, we noticed recently numerous reports on the incidence of urochromogen reaction in a large number of tuberculous individuals. In fact, according to some investigators, the reaction was even fine enough to permit following the progress of the treatment. As in our preliminary work[17] with this method we were not able fully to substantiate the optimistic findings of other workers, we decided to repeat the work in a larger number of cases.

Dombrowski[18] in 1907 determined the urochrome of the urine in health and disease. By his method of determination with copper, he found that in health there is excreted 0.3885 gm. in a twenty-four-hour specimen of urine, whereas in pneumonia and in typhoid fever this amount may be increased to 1.0572 gm. per day. Four years later Weisz[19] pointed out certain discrepancies in Dombrowski's results. According to this author the urochrome of Dombrowski is not identical with the normal urinary urochrome. On isolating this coloring matter from normal and pathological urines, he found that the cadmium salt of Dombrowski urochrome is insoluble in diluted acetic acid, whereas the normal urochrome is soluble in this acid.

According to Weisz, the normal coloring matter of the urine is derived from a chromogen which in its properties is similar to the proteic acids which are present in the urine. This urochromogen when present in the urine is the substance which is responsible for the Ehrlich diazo reaction. Again, according to Weisz[20] there are two distinct urochromogens, which he distinguishes as A and B. The B urochromogen gives the diazo reaction promptly. The A urochromogen, on the other hand, does not give this reaction in freshly voided urines. When, however, these urines are allowed to stand in the incubator for twenty-four hours or so, a positive diazo reaction will be given by the A urochromogen.

When these urochromogens are oxidized either by dilute solution of potassium permanganate or by iodin and potassium thiosulphate, they are changed to the true urochromogen of the urine.

In 1911 Weisz[21] reported the results of his six years' study of the urochromogen reaction in urines of patients suffering with pulmonary tuberculosis. He found that the test for urochromogen in these patients was positive in the great majority of cases. It is necessary, he said, in considering the diagnostic significance of this test, to exclude those other diseases which also yield a positive Weisz reaction. In this class he groups typhoid fever, measles, scarlet fever, septicemia, surgical tuberculosis, etc. He also found

17. Bronfenbrenner, Rockman and Mitchell: Biochem. Bull., 1915, xiii, 80.
18. Dombrowski: Compt. rend. Acad. d. Sc., 1907, cxlv, 575; Ztschr. f. physiol. Chem., 1908, liv, 390.
19. Weisz, M.: Biochem. Ztschr., 1911, xxx, 333.
20. Weisz, M.: Med. Klin., 1911, vi, 1661.
21. Weisz, M.: Munchen. med. Wchnschr., 1911, lviii, 1378.

that patients who had been narcotized, or who had received injections of tuberculin, gave positive results with this reaction. A positive reaction in tuberculosis may, according to Weisz, indicate either a low resistance to the tuberculous toxin, or a spreading lesion with an increasing production of toxin. A positive reaction is of unfavorable prognostic significance.

The destruction of tissue causing the excretion of the urochromogen also causes an increase in the output of neutral sulphur of the urine. The oxyproteic acid fractions with which the urochromogen seems to be closely related is normally present in the urine in small quantities. According to Bondzynski[22] and his pupils antoxyproteic acid is a mixture of the proteic acid with urochromogen.[23]

The destruction of tissue which liberates the increased quantities of proteic acids can be measured in several ways. These substances contain nitrogen and sulphur and are combined with urochromogen. Methods have been advanced, therefore, to study this substance, either by precipitation and nitrogen determination, or by precipitation and sulphur analysis, or by the urochromogen method.[24]

Paranhos and Grolito,[25] found that the Weisz reaction was positive in 90 per cent. of tuberculous patients. Cotton[26] obtained excellent results. Vitri[27] of Buenos Aires obtained positive reactions in patients in whom the tuberculin skin test was positive. He found that the presence of a little albumin, sugar or urobilin did not interfere with the Weisz reaction. Dozzi[28] as well as Pignacca[29] consider this test pathognomonic of tuberculosis. Curti[30] found that this test was positive in pneumothorax as well as in all cases of clinical tuberculosis. The reaction, according to this author, ran parallel with the clinical symptoms of the disease and became negative in cases which were, clinically, considered cured. Tecon and Aimart,[31] Jaquerod,[32] Kaplansky,[33] Puley[34] and others, have found this reaction of value. Similar results were obtained by Levy,[35] Heflebower,[36] Metzger and Watson,[37] Ferrannini,[38] Bruni,[39] Burgess[40] and Sinclair.[41]

---

22. Bondzynski: Kosmos, 1911, xxxv, 650.
23. Sklepinsky, A.: Pharm. Centralbl., 1911, lii, 615.
24. In fact, according to Weisz the interdependence between the appearance of excess of neutral sulphur in the urine and the incidence of urochromogen reaction is so great that he is inclined to take the positive outcome of urochromogen test for an indicator of the presence of excess of neutral sulphur in the urine. It is in this sense that we identified in our first paper (Bronfenbrenner, Rockman, and Mitchell, Biochem. Bull., 1915, No. 13, p. 80) the findings of urochromogen with the sulphur, although the Weisz reaction per se is, of course, not the test for neutral sulphur, but for urochromogen.
25. Paranhos and Grolito, V.: Brazil med., 1913, xxvii, 101.
26. Cotton, E.: Rev. méd. de la Suisse romande, 1913, xxxiii, 217.
27. Vitri, G.: Semana méd., 1913, xx, 110.
28. Dozzi, L.: Gazz. d. osp., 1913, xxxiv, 815.
29. Pignacca, G.: Gazz. d. osp., 1914, xxxv, Nos. 34-35.
30. Curti: Quoted in München. med. Wchnschr., 1914, p. 1577.
31. Tecon and Aimart: Presse méd., 1914, p. 135.
32. Jaquerod: Discussion, Presse méd., 1914, p. 135.
33. Kaplansky: Discussion, Presse méd., 1914, p. 135.
34. Puley: München med. Wchnschr., 1915, xxvi, 1009.
35. Levy: Deutsch. med. Wchnschr., 1915, xli, 1212.
36. Heflebower: Am. Jour. Med. Sc., 1912, cxliii, 221.
37. Metzger and Watson: Jour. Am. Med. Assn., 1914, lxii, 1886.
38. Ferrannini: Riforma med., 1915, xxxi, 479.
39. Bruni: Gazz. d. osp., 1915, xxxvi, 401.
40. Burgess: Jour. Am. Med. Assn., 1916, lxvi, 82.
41. Sinclair: Jour. Am. Med. Assn., 1916, lxvi, 247.

The technic of the Weisz reaction is very simple. Several cubic centimeters of urine are diluted two or three or more times to reduce the intensity of the color of the urine. To this are then added several drops of a 0.1 per cent. solution of potassium permanganate. In the presence of the urochromogen a deep yellow color appears.

All told we examined 1,046 cases, of which number 681 were the conditions in which the increased output of neutral sulphur was not suspected, and the remaining 365 cases were classified as follows:

TABLE 5.—Reactions in Various Diseases

	Number of Cases	Diazo		Weisz		Percentage Positive	
		Pos.	Neg.	Pos.	Neg.	Diazo	Weisz
Typhoid	67	32	35	18	49	47.7	26.9
Diabetes	58	1	57	9	49	1.7	15.5
Cancer	67	0	67	4	63	0	5.9
Tuberculosis	173	14	159	22	151	8.0	12.7
Unclassified	681	43	638	68	613	6.3	9.9
Total	1,045	90	956	121	925	8.6	11.5

From these results we are led to the conclusion that the Weisz reaction is not always incident in diseases characterized by tissue destruction. In 365 cases of such diseases which are characterized by marked protein katabolism, only fifty-three positive urochromogen reactions were obtained.

Moreover, when present, this reaction is not pathognomonic of tuberculosis; in fact, as the table shows, the percentage of incidence of urochromogen reaction is even higher in diabetes, and especially in typhoid, than in tuberculosis. The high incidence of urochromogen reaction in different pathological conditions was shown before in cancer,[42] diabetes,[43] pneumonia,[44] cachexia,[45] and in typhoid.[46] For instance, Halbey[47] found that the urochromogen test was positive in nine cases of typhoid at each application of the test so long as the fever kept up. It was positive in all cases of measles, scarlet fever,

42. Goodridge, F. G., and Kahn, M.: Biochem. Bull., 1915, iv, 118.
43. Einhorn, M., Kahn, M., and Rosenbloom, J.: Arch. f. Verdauungskr., 1911, xvii, 557.
44. Mancini: Deutsch. Arch. f. klin. Med., 1911, ciii, 288.
45. Mazzitelli: Riforma med., 1912, xxviii; Abstr. Jour. Am. Med. Assn. 1913, lix, 978.
46. Kuhl, A.: Deutsch. med. Wchnschr., 1915, xxxi, 912.
47. Halbey, K.: Med. Klin., 1915, xi, 830.

erysipelas and malaria. It was constantly negative, on the other hand, in seventeen cases of pulmonary disease, even in advanced forms, and negative, likewise, with miliary tuberculosis. A positive reaction was thus obtained in 33 per cent. of the fifty-two patients tested.

The high figures for positive Weisz reaction that have been reported by a number of observers in various diseases and especially in tuberculosis, may be due to the fact that atypical color reactions were considered as indicative of a positive test. When dilute potassium permanganate solution is added to the urine there is frequently noticed a pinkish tinge, which, unless care is taken and a standard prepared with which comparisons can be made, may be erroneously interpreted as an indication of the presence of urochromogen.

It is evident from the results reported in Table 5 that the urinary examination cannot be used as a safe control in serum diagnosis of tuberculosis. For, on the one hand, the urochromogen reaction is found to be positive only in about 13 per cent of cases of tuberculosis, in which the serum reaction is positive usually in about 92 per cent of cases, and, on the other hand, the frequent occurrence of this reaction in conditions other than tuberculosis makes valueless the findings of 10 per cent. of positive reactions in nonclassified cases, as far as diagnosis of tuberculosis is concerned. The actual relationship between the two reactions, however, can be judged only by performing both tests in the same cases. This we did in 212 cases, with the results shown in Table 6.

TABLE 6.—Comparison of Urochromogen and Serum Reactions

			Serum Test	Tub. Bacilli Present	Weisz Reaction Present	Percentage of Occurrence	
						Serum	Weisz
Lung tuberculosis	Stage I....	Serum test positive..	21	1	1	84 *	4
		Serum test negative..	4	..	..		
	Stage II...	Serum test positive..	31	15	1	94	9.4
		Serum test negative..	2	2	2		
	Stage III..	Serum test positive..	2	2	..	15.3	30.7
		Serum test negative..	11	11	4		
No tuberculosis clinically.....		Serum test positive..	7	0	3	4.9	7.8
		Serum test negative..	134	0	8		

* We do not offer at present any definite explanation for the comparatively low number of positive findings in this series, but it is evident that at least three causes could well explain it: (1) the mistaken diagnosis; (2) absence of resistance on the part of the patient (Bronfenbrenner, J.: Science, 1914, xxxix, 804; also Proc. Soc. Exper. Biol. and Med., 1914, xi, 92); (3) the known fluctuation in the antibody content in the blood of recently infected individuals (see discussion by Meyer, Deutsch. med. Wchnschr., 1914, p. 513).

The examination of Table 6 reveals the fact that the urinary findings do not go parallel with serum findings. On the contrary, whereas the serum findings, very high in primary and secondary stages of pulmonary tuberculosis, falling very markedly in the tertiary stage of the disease, the urinary findings seem to show a marked increase in frequency as the disease progresses.

Although as Table 5 has shown, the katabolic changes in the body are not always followed by the presence of urochromogen in the urine, it is evident that there is undoubtedly an increased incidence of urochromogen reaction in later stages of the destructive process as compared with the earlier stages of the same disease. This increase in the incidence of Weisz reaction together with the corresponding inverse changes in the occurrence of the serum test, may be of great value in confirming our previous conclusion that cases of clinical tuberculosis in advanced stages in which the serum reaction is negative are characterized by the loss of resistance on the part of the patient.[17] In these cases the destructive processes develop more rapidly, which is indicated by the large excess of neutral sulphur in the urine, as well as by a high percentage of positive urochromogen findings. As for the seven cases of positive serum findings out of the total of 141 cases in which tuberculosis was not suspected, the urinary analysis does not offer any possible way of controlling the diagnostic value of these findings. As we have mentioned before, however, we do not feel inclined to attribute such findings to the possibility of nonspecific fixation, but rather to the fact that in its earlier stages the tuberculous process does not induce symptoms sufficient for clinical diagnosis. This is why a number of cases may present at necropsy unmistakable symptoms of a tuberculous process having taken place at some time during the life of the person without his being aware of the presence of the disease.

CONCLUSIONS

Attempts to study further the value of biological methods of diagnosis of tuberculosis led us to the following conclusions:

Different samples of tuberculin of Besredka, though apparently identical in the mode of their preparation, may differ among themselves in their specific values.

The most striking variation is in the amount of lipins contained in tuberculin.

It is necessary to free each sample of tuberculin of all its lipin fraction before using such tuberculin for the complement deviation test.

The lipins may be extracted by fat solvents, but the easiest method was found to be that of separation of the protein fraction by precipitation.

Precipitation of the antigenic fraction of tuberculin also offers the possibility of using a standard number of units of antigen and thus eliminating variations due to the quantitative differences in specific properties of different samples of tuberculin, without increasing the chance of obtaining lipotropic reactions.

It seems, however, that different samples of tuberculin may vary also qualitatively.

The variation rests apparently on the fact of the existence of strain specificity in the antibody.

The existence of strain specificity in tuberculosis may explain why the results obtained by different investigators in the complement deviation test for diagnosis of tuberculosis vary so much.

The tuberculin of Besredka seems to give the best results in diagnosis by the complement deviation test.

Even though the test is positive in a certain number of clinically non-tuberculous cases, the reaction seems to be specific.

In at least 87 per cent. of such cases the fixation was obtained also with one or more preparations of tuberculins other than that of Besredka.

The attempt to control serum findings by the urinary examination for urochromogen was not successful in general, because we were unable to confirm the frequent occurrence of the Weisz reaction in tuberculosis. A comparison of the frequency of occurrence of the two reactions in different stages of the disease suggests that negative serum findings in the face of the positive Weisz reaction may have an unfavorable prognostic significance.

ADDENDUM

Since the above material was reported, our attention has been called to the article of Craig (*American Journal of the Medical Sciences*, 1915, cl, 781), in which the author gives a simple method of preparation of antigen for the complement deviation test for the diagnosis of tuberculosis. The results obtained by Craig are in general in accord with those obtained by us with the antigen of Besredka. Indeed, they are even more encouraging than ours.

In one respect, however, our respective results seem to differ in a marked degree; that is, in the percentage of positive reactions obtained among syphilitics. In our series about 20 per cent. of all syphilitics reacted with the antigen of Besredka, whereas in the series of Craig, the occurrence of tuberculosis among syphilitics as judged by this test was less than 1 per cent. As we stated at the time, the comparatively high percentage of positive reactions among syphilitics in our series was not due to nonspecific fixations. In fact, in cases in which such study was undertaken, we invariably found the two specific antibodies coexisting independently. We came to the conclusion, therefore, that the comparatively frequent simultaneous occurrence of the two reactions is due to the fact that tuberculosis is more frequent among syphilitics than among the persons free from luetic infection. It seems that syphilitic infection makes the person less resistant to a subsequent infection with tuberculosis, or at least renders him less resistant against the progress of tuberculosis previously contracted. Such a conclusion is apparently borne out by

clinical observations of such men as Fournier, C. F. Marshall, Douty, F. H. Andrews, Sir Jonathan Hutchinson, and others (see *Oxford System of Syphilis*, 1914, iii, 197).

The fact, however, that in the series of 150 syphilitics Craig obtained a positive complement fixation with his tuberculin in only two cases, speaks at the first glance against such a conclusion. But if one takes into consideration that the material of Craig must have consisted largely or entirely of strong young men of military age, the difference can be explained easily. In our series of syphilitics we dealt with men or women of all ages, mostly working people, greatly exposed to infection with tuberculosis in their factory surroundings, and even more so in the unhealthy conditions of their life in the slums. Craig's material consisted of a group of men who must have been comparatively free from tuberculosis at the time of admission to military service, and who, in addition, having been admitted to service, were placed in conditions of life which are exceptionally favorable for assisting the person in resisting any possible preexisting tuberculous infection and sheltering him from any possible new infection. We therefore think that our previous statement about the frequency of occurrence of tuberculosis among syphilitics is correct, if the syphilitics are taken from a group of persons the same as that from which the rest of our material was derived.

Western Pennsylvania Hospital.

TWO ADDITIONAL CASES OF MILIARY TUBERCULOSIS OF THE PLACENTA WITH CLINICALLY LATENT TUBERCULOSIS OF THE MOTHER*

CARL VERNON WELLER, M.D.
Instructor in Pathology, University of Michigan
ANN ARBOR, MICH.

The two cases of placental tuberculosis which form the subject of this report are of unusual interest, for in each the disease was so slightly active as to be unrecognized clinically in the mother, but was found in the course of the routine microscopical examination of the placenta. In one instance no history suggestive of tuberculosis could be elicited and the disease, apparently incipient during pregnancy, has become latent since the birth of the child. This case, therefore, is similar to the one reported from this laboratory in 1913 by Dr. Warthin,[1] in connection with which he at that time called attention to the increased importance of the sociologic and eugenic aspects of the transmission of tuberculosis from parent to child, provided that it can be shown that in women who have incipient or clinically unrecognizable tuberculosis the bacilli may enter the blood stream and cause a miliary tuberculosis of the placenta or congenital infection of the fetus.

The second case to be reported is in some respects even more unique. In this instance, also, the maternal tuberculosis was clinically unrecognizable at the time of admission to the hospital and was found only through the discovery of miliary tubercles in the course of the routine examination of the placenta. This patient, however, had wilfully falsified in regard to her history in order to conceal an earlier diagnosis of tuberculosis. This she admitted when she was told of the finding of placental tuberculosis. Unlike the first case and the one recently reported by Dr. Warthin, this woman's tuberculosis became more active after the birth of the child and she returned to the hospital with unmistakable clinical signs of pulmonary tuberculosis.

Without including here a review of the literature, attention is called to the fact that the reported cases of placental tuberculosis may be arranged in three groups:

1. Cases in which the mother was known to be tuberculous and in which, as a result of this knowledge, the placenta was given intensive

* Submitted for Publication Jan. 28, 1916.
* From the Pathological Laboratory of the University of Michigan.

1. Warthin, A. S.: Miliary Tuberculosis of the Placenta, Jour. Am. Med. Assn., 1913, lxi, 1951.

study and found to exhibit the characteristic lesions of tuberculosis, either with or without a demonstration of the bacilli.

2. Cases in which the mother was known to be tuberculous and in which the presence of tubercle bacilli in the placenta or the fetal blood was demonstrated by staining methods or by animal inoculation without the recognition of the lesions of tuberculosis in the placenta. One must believe that in many of these cases the failure to find lesions was due either to unfamiliarity with the somewhat varied microscopical picture of placental tuberculosis, or to the fact that the amount of placental tissue examined in section under the microscope was such a small proportion of the total organ that characteristic lesions were not included.

3. Cases in which the maternal tuberculosis was clinically unrecognizable and in which characteristic histopathologic lesions of tuberculosis were found in the routine examination of the placentas. In this group fall the two cases forming the subject of this report, the only other case in the group being the one reported by Dr. Wàrthin, to which reference has been made.

That three such cases should be found in one laboratory and within the space of three years is strong additional argument, not alone for the belief that the maternal transmission of tuberculosis is much more common than is ordinarily taught, but also for the little recognized fact that a "subclinical" tuberculosis may be so influenced by pregnancy that bacilli may enter the blood stream and produce placental or fetal miliary tuberculosis.

REPORT OF CASES

Summaries of the histories of the two cases here reported follow:

CASE 1.—Miss K., aged 21, entered the maternity ward of the University Hospital, service of Dr. Reuben Peterson, April 23, 1914. The patient was then in about the eighth month of pregnancy. There was no history of tuberculosis in her family, father, mother and eight brothers and sisters being alive and well. One sister died in childbirth. On physical examination by Dr. Seeley the patient was found to be of slight build but fairly well nourished. No signs of any pulmonary lesion were discovered and the examination of the heart was likewise negative. The findings in the routine ante partum obstetrical examination do not concern us here. The Wassermann reaction was reported negative by one serologist and positive (one plus) by another. Blood and urinary findings were negative.

June 11, the patient was delivered by Dr. Bartholomew of an apparently healthy female child. The puerperium was uneventful and the patient returned to her home June 27. The child was left at the University Hospital until July 16. It was then 35 days old, had gained 550 gm., and was apparently normal in all respects.

The placenta from this birth was placed in formol after being securely tagged with the name of the mother. It was sent to the pathological laboratory and in the course of the routine microscopical examination miliary tubercles were found. These lesions will be described later.

In October, 1915, the family physician of this patient, replying to an inquiry addressed to him by Dr. Loomis, said that both mother and child were under observation, were apparently well and had given no evidence of tuberculosis at any time.

CASE 2.—Mrs. C., aged 27, was admitted to the obstetrical wards of the University Hospital, service of Dr. Peterson, Jan. 28, 1914. She was then in the ninth month of pregnancy. The patient denied any family history of tuberculosis, saying that her father, mother, three brothers and two sisters were living and well. Two sisters died in childhood. Concerning her personal history she said that she had never been strong until the past few years, but that she had made a good recovery from measles and scarlet fever in childhood and also from "congestion of lungs and bowels" five years previously. The patient was married nine years before and had one child, aged 7. There had been no other pregnancies.

The history of the present pregnancy presented nothing unusual. There had been considerable nausea and vomiting in the second and third months, but little afterward. No eye symptoms or dizziness, no swelling of ankles, no headache had occurred.

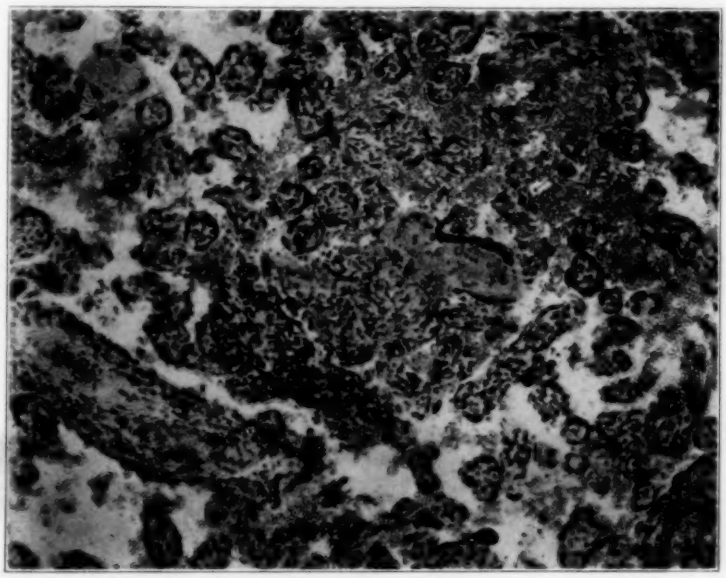


Fig. 1.—Miliary tuberculosis of the placenta. Clinically latent tuberculosis of the mother. In the center of the field an intervillous epithelioid tubercle.

On physical examination, made by Dr. Loomis Jan. 30, 1914, the patient was found to be of a rather large frame, with musculature and panniculus good. Face pale, but conjunctivae and lips of good color. Eyes reacted normally to light and accommodation. Neck, mouth and throat were negative. The thorax presented marked infra- and supraclavicular fossae and was found to be somewhat larger, and to move more freely on the left than on the right side. The lung borders had a wide excursion. Resonance was normal. The breath sounds were likewise normal except for occasional piping râles, and the voice sounds were generally, but uniformly, increased. It was noted that the patient had some cough, and accordingly fine crackling râles were carefully sought for but were not heard. Auscultation and percussion of the back gave only negative findings.

The routine antepartum examination of breasts, abdomen and pelvis need not be detailed. There was no indication that the confinement would be otherwise than normal. Such proved to be the case, and Mrs. C. was delivered rather precipitately Feb. 4, 1914. The child was an apparently healthy male weighing 7 pounds, 1 ounce. The puerperium was normal throughout and the history of the child was uneventful during the fourteen days that it remained in the hospital. At the expiration of this period the mother took the child to her home in a distant part of the state.

The placenta from this birth was placed in 10 per cent. formaldehyd solution at once and delivered to the pathological laboratory March 8, 1914. In the routine microscopical examination of this placenta there was found a group of four or five miliary tubercles. The microscopical findings will be described in greater detail later.

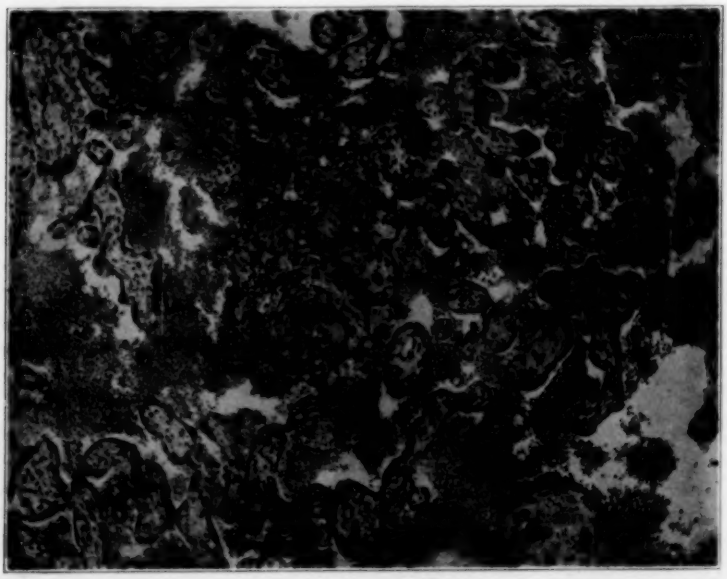


Fig. 2.—Miliary tuberculosis of the placenta, Case 2. Pulmonary lesion unrecognizable during pregnancy, but becoming active after birth of child. An intervillous epithelioid and giant-cell miliary tubercle.

In January, 1915, letters of inquiry were sent to Mrs. C. and to her home physician asking for additional information as to a possible history of tuberculosis (although the patient had stated that there was no history of tuberculosis in the family), and also in regard to the condition of both mother and child. The reply of the mother was especially interesting, throwing, as it did, much light on the subsequent history of the case and revealing an altogether too common attitude on the part of the patient. Mrs. C.'s letter follows in part:

". . . I am very glad to be able to send you a favorable report; also glad to know you are interested enough in us to write and inquire. Baby is strong and well and has never been sick enough to call a doctor . . .

"As to my own health, I am well and strong as can be. I have had perfect health ever since I left the hospital. I understand that Dr. Peterson wrote to our doctor here, saying that at the time of the baby's birth you found signs of tuberculosis. I am not at all surprised as I have been told before that I had tuberculosis but I did not believe it, so thought I would not speak of it and *let you find it if you could*. I lay our good health all to fresh air, for we are fresh air fiends . . ."

The patient's home physician kindly wrote under date of Jan. 19, 1915, saying that Mrs. C. had a history of tuberculosis dating back some eight or ten years. He had not had an opportunity to examine mother or child since her confinement, but as far as he could learn they were apparently well. Five months later (June 30, 1915) her physician again wrote, saying that Mrs. C. was losing weight rapidly and apparently doing badly, although no active pulmonary focus was discovered. The baby remained in "excellent condition." Because of this information the patient was advised to return for examination, which she did July 26, 1915, entering as an outpatient, the service of Dr. A. W. Hewlett, through whose kindness the notes taken at that time were placed at my disposal. The patient then disclosed her earlier history of pulmonary tuberculosis. She said that she had had pneumonia in the right lung six years previously, after which she did not recover strength and was told that she had tuberculosis. Accordingly she went West for three years, but returned apparently perfectly well.

In the seventeen months which had elapsed since the birth of her child, the patient had been well, except for some cough and sputum, until the beginning of the summer, when she began to grow weak and tired and to raise more sputum. The following April she had pain in the right upper chest, at some times dull and radiating to the scapular region, and at other times knife-like in character, keeping her awake at night. She had been taking her own temperature at 4 p. m. each day but had had no fever except for a period of about one month, when she had noted an afternoon temperature 2 to 3 degrees above normal. During that month she was very tired so that she had to go to bed most of the time, but more recently she had felt stronger. There had been a loss of weight of 20 pounds in the previous six months.

In examining the patient at this admission, Dr. Hewlett found increased fremitus in the right scapular region and beneath the inner end of the right clavicle. Sharp crackling râles, brought out by coughing, were occasionally heard in the interscapular region. A diagnosis of pulmonary tuberculosis was made.

The lesions discovered microscopically in the placentas of these two patients are in every respect characteristic and there can be no doubt that they are miliary tubercles. Without a detailed discussion of the histopathology of placental tuberculosis (which may be found in the papers of Schmorl and Geipel[2] and Warthin[3]), attention is called to the fact that the differential diagnosis of placental tubercles presents but few difficulties if the lesions are present in at least moderate numbers and have not reached a stage of advanced healing. This point has been emphasized by Warthin,[1] and he has shown that even when the tubercles have advanced to a healing stage the two other things which need to be considered in differentiation are essentially unlike them. In small localized areas of *syphilitic chorionitis* the process involves only the stroma

2. Schmorl and Geipel: München. med. Wchnschr., 1904, li, 1676.
3. Warthin, A. S.: Jour. Infect. Dis., 1907, iv, 347.

of the villi and the villi are not fused into a solid fibroblastic or fibroid mass. In the small *healing infarct* the villi may be fused, but there is no fibroblastic formation, or only the slightest, between them. In the *healing tubercle,* on the other hand, if villi are fused in the mass, there is an abundant intervillous epithelioid or fibroblastic proliferation.

MICROSCOPICAL FINDINGS

The original sections made in the routine examination of *Case 1* each showed four or five young and active miliary tubercles. Examination of other sections has shown that the distribution varies somewhat, but no section examined from this case has failed to disclose at least one tubercle. These tubercles are practically all in about the same stage, showing an active epithelioid proliferation without caseation, although some of the larger ones reveal a slight karyorrhexis and diffusion of chromatin. Giant cell formation was not noted. In regard to location, a large majority of the tubercles are intervillous, often involving within the mass four or five villi the outlines of which may still be made out around the periphery of the tubercle. Fewer intravillous tubercles are found and these correspond in their young epithelioid character to those just described. Earlier stages were not found, so that the bacilli must have been given off some time before delivery, and most of them at about the same time.

The original section from which the diagnosis of placental tuberculosis was made in *Case 2* showed a group of four or five intervillous miliary tubercles which were epithelioid in character. Study of a large number of sections from this case shows the total number of tubercles to be much smaller than in Case 1, many sections containing none, and but rarely more than one to a section being found. Although various stages are present, in general these tubercles are somewhat older than those of Case 1. None, however, show healing, but typical Langhans' giant cells are frequently seen, and in some instances there is an early fibroblastic proliferation. As in the first case, intervillous tubercles greatly predominate over those of other locations.

It is to be regretted that it is technically impossible to demonstrate tubercle bacilli in the lesions in these placentas. They were both intensely fixed in formol, which always makes the differential staining of the tubercle bacillus very difficult and often impossible. We are not alone in this experience as it is mentioned in various works on microscopic technic and constitutes one of the many objections to the routine use of formol as a fixing agent. We believe, however, that our inability to demonstrate the bacilli does not in the least detract from the value of these cases, for the lesions are absolutely characteristic; they differ in no respect from similar lesions in other cases in which the bacilli have been demonstrated in large numbers; and in one of the two cases here presented, the diagnosis of tuberculosis has been supported in an unusual way by the subsequent development in the mother of an active pulmonary process.

As far as can be ascertained, there has been no evidence of tuberculosis in either of the children from these births. Whether this is due to the fact that the bacilli were of relative slight virulence, or that they

passed the placenta in too small numbers to determine a clinically recognizable infection, or whether the fetal tissues offered more than ordinary resistance, can only be conjectured.

SUMMARY

The two cases of placental tuberculosis here presented were discovered in the routine microscopical examination of the placentas. The mothers did not have clinically recognizable lesions at the time of delivery, but in one case an active pulmonary tuberculosis later developed. The other case became latent after the birth of the child. These cases are additional proof of the fact that even an incipient and unrecognizable tuberculosis may be so influenced by pregnancy as to cause a placental, and therefore potentially a fetal, miliary tuberculosis.

My thanks are due Dr. A. S. Warthin, who made the original diagnosis of placental tuberculosis in these cases, for placing the material at my disposal; to Dr. Reuben Peterson and Dr. A. W. Hewlett for the use of the records of their respective departments; and to Dr. F. L. Loomis of the Department of Obstetrics and Gynecology for his assistance in following up the after-history of the cases.

PROGRESSIVE LIPODYSTROPHY *

CHARLES HERRMAN, M.D.
Attending Pediatrist to the Lebanon Hospital
NEW YORK

A few years ago I had under observation at different times two girls aged 10 and 12 years, respectively, in whom I was struck by the marked contrast between the emaciated face with hollow cheeks and the good development of the rest of the body. These children were brought to the clinic for slight ailments and were not affected with any constitutional disease. I realized that I was dealing with a rare condition, but was at a loss to know where to search for a description of it in the literature. A paper recently published by Feer[1] on "Lipodystrophia Progressiva" immediately cleared up the diagnosis. Unfortunately no detailed history, photographs or measurements were taken at the time, and the patients are no longer under observation.

I had the good fortune recently to discover a typical example of this condition in the person of a mother who brought her children for treatment.. The history is briefly as follows:

REPORT OF CASE

History.—Mrs. K., aged 32, Hebrew, has been married for ten years to her cousin. She had three brothers and two sisters. One sister died in childhood of pneumonia. One brother and the sister are married and have healthy children. No other member of the family has had a similar condition; neither the mother of the patient nor the other female members were unusually fat or thin, nor did any one have an increased amount of adipose tissue in the lower part of the body. There is no family history of syphilis, tuberculosis, alcoholism, diabetes or nervous diseases. The birth was normal, the patient was breast fed, and her physical and mental development during childhood was normal. At the age of 3 she had measles without complications. Since that time, with the exception of occasional headaches, her health has always been good. The change in her face was first noticed at the age of 6. It began insidiously, without any fever, pain or discomfort, and gradually became more marked, so that at 11 the fat of the face had almost entirely disappeared. Later the dystrophy spread to the neck, upper part of the chest and arms, so that these parts became distinctly thinner. When she was 11 years old the family emigrated from Russia to this country. The patient was taken to a well known New York physician, who after a careful examination told the mother that he hoped her other children were as healthy. The patient began to menstruate at 13 years. She married at 22 years; her weight at that time was 44.5 kg. (98 pounds). After her marriage she began to notice that the lower part of her body was growing stouter, and this continued, so that at the age of 28 she weighed 53.5 kg. (117 pounds) and at 31 years 55.5 kg. (121 pounds). Except for the disfiguring appearance of her face, she has not suffered in any way, her appetite is good, her

* Submitted for publication Jan. 27, 1916.

1. Feer: Lipodystrophia Progressiva, Jahrb. f. Kinderh., 1915, lxxxii, 1.

bowels regular, she has no disturbances of sleep, no palpitation or nervous symptoms. She has two children, a girl aged 8½ years, who is perfectly healthy, and a boy aged 7 years, who has chronic endocarditis following an attack of rheumatism. She has had no miscarriages or stillbirths, and is now pregnant for the third time. Both children were breast fed for one year.

Physical Examination.—Measurements: weight, before pregnant, 55.5 kg.; in seventh month of pregnancy, 59 kg.; height, 150 cm.; circumference of the head, 53 cm.; neck, 31.5 cm.; chest, 80 cm.; arm, 22 cm.; elbow, 21.5 cm.; forearm, 21.5 cm.; thigh (at level of great trochanter), 62 cm.; at 15 cm. above patella, 53.5 cm.; knee, 36.5 cm.; leg, 37.5 cm.; ankle, 23 cm.; thickness of the raised fold of fat and skin—cheek, 5 mm.; breast (over nipple), 6 mm. axilla (level of nipple), 6 mm., interscapular region, 8 mm.; arm, 5 mm.; forearm, 5 mm.; neck, 7 mm.; thigh, 45 mm.; leg, 27 mm.

One is immediately struck by the disproportion between the upper and lower parts of the body. The face is haggard, and has a cadaverous expression (Fig. 1). It is hollowed in the temporal region, the malar bones are prominent, and below them there are two hollows, an upper small one, and a lower large one

Fig. 1.—Progressive lipodystrophy in a woman of 32 years. Author's case

separated by a thin bridge, corresponding to the zygomatic muscle. This renders the masseter more distinct, and gives a fulness around the corners of the mouth. There are also distinct grooves under the lower lip and on either side of the chin. When the patient smiles, folds form on both sides of the mouth. The neck shows a marked reduction of the subcutaneous fat; also the arms and upper part of the trunk. There is no disturbance of function or sensation in these parts. Examination of the hair, eyes, ears, nose, throat and teeth shows nothing abnormal. The patient does not complain of flushing, profuse perspiration or chilliness. The breasts are well developed, and on account of the small amount of subcutaneous fat are unusually hard. Examination of the heart and lungs shows normal conditions. From the crests of the ilia downward there is a very marked increase in adipose tissue; this is most distinct in the gluteal region and on the outer side of the thighs. It is less distinct on the leg, and the ankle is not at all enlarged. The function of the muscles, sensation and the tendon reflexes are normal. Examination of the blood and urine shows nothing abnormal. The roentgenologic examination of the arm and of the skull (sella turcica) shows nothing pathological.

Number of Cases, Race, Sex.—Up ti the present, fifteen cases of progressive lipodystrophy have been reported by the following authors: Barraquer,[2] Campbell,[3] Holländer,[4] Simons,[5] Laignel-Lavastine and Viard,[6] Cohn,[7] Lewandowsky,[8] Weber,[9] Christiansen,[10] Feer,[1] and Pic and Gardère.[11] In most of these reports a detailed history and measurements are not given. Three cases in males which are somewhat doubtful are reported by Shaw,[12] Hertz and Johnson,[13] Husler.[14] The condition is probably not as rare, however, as the figures would seem to indicate, for like other uncommon conditions, it is sometimes not recognized because not known. A number of the patients were Hebrew, though there does not seem to be any marked predisposition of the race to this condition. All of the undoubted cases have been in females; but it is not at all certain that males may not be occasionally affected.

Etiology and Pathogenesis.—In those few instances in which the family history is given, there is no indication of any inherited tendency, either to the same condition or to a disturbance of the fat metabolism. Syphilis, tuberculosis and alcoholism apparently play no part in the causation of this condition. In those patients in whom the Wassermann test was made the result was uniformly negative. The patients may of course be affected with intercurrent diseases, but these apparently bear no relation to the underlying condition, which appears to be a form of trophoneurosis. One naturally thinks of some disturbance in the function of one or more of the ductless glands, but symptoms of hyperthyroidism such as palpitation, tremor, tachycardia, those of hypothyroidism, such as mental and physical sluggishness, subnormal temperature, constipation, are absent. Symptoms pointing to a disturbed function of the pituitary, suprarenals or ovaries are likewise absent. The condition occurs in married and in unmarried women; they present no unusual disturbances of menstruation, pregnancy or lactation. In addition, none of the patients has received any benefit from the administration of the extracts of the ductless glands. It is not unlikely that

2. Barraquer: Abstr. Neurol. Centralbl., 1907, p. 1072.
3. Campbell: Tr. Clin. Soc. London, 1907, xl, 272; Proc. Roy. Soc. Med., London, 1913, vi, 71.
4. Holländer: Ztschr. f. d. ges. Neurol. u. Psychiat., 1911, v, 633.
5. Simons: Ztschr. f. d. ges. Neurol. u. Psychiat., 1911, v, 29; *Ibid.*, 1913, xix, 377; Ztschr. f. Kinderh., 1915, xi.
6. Laignel-Lavastine and Viard: Nouv. iconog. de la Salpétrière, 1912, xxv, 473.
7. Cohn: Abstr. Neurol. Centralbl., 1913, xxxii, 779.
8. Neurol. Centralbl., 1913, xxxii, 866.
9. Weber: Brit. Med. Jour., 1913, p. 1154.
10. Christiansen: Mentioned by Feer, Note 1.
11. Pic and Gardère: Lyon méd., 1909, cxiii, 61.
12. Shaw: Tr. Clin. Soc. London., 1905, xxxviii, 222.
13. Hertz and Johnson: Proc. Roy. Soc. Med., London, 1913, vi, 92.
14. Husler: Ztschr. f. Kinderh., 1914, x, 116.

something in the circulating blood is the causative factor. The symmetrical, bilateral character would seem to indicate this. The fact that only certain parts of the body are affected, would not be a strong argument against such an assumption, for it is well known that certain organisms, toxins and other substances have a special affinity for certain tissues; and it is not impossible to conceive of a substance that would cause a diminution or disappearance of the fat in one part of the body and an increase in another part. By injecting the blood of patients in

Fig. 2.—Progressive lipodystrophy in a woman of 21 years. Simons' case.

the *active progressive stage* of the disease into monkeys it might be possible to produce somewhat similar changes.

Symptoms and Course.—The loss of the subcutaneous fat of the face begins insidiously without fever, pain or other symptoms, and gradually becomes more marked; it is bilateral and symmetrical. In six of the patients, including my own, the disease began between the fifth and the seventh year; in the remainder, about the time of puberty. In the majority of the cases the loss of subcutaneous fat then extended

to the neck, arms and upper part of the trunk. The orbital fat is little if at all affected and as no postmortem examinations have been made it is still unknown whether the internal fat is diminished. The increase of adipose tissue in the lower part of the body begins or becomes more marked at the time of puberty. This increase is most distinct in the gluteal region and on the outer side of the thigh, but also involves the rest of the leg. As a rule, both sides are equally affected; occasionally the increase is greater on one side. The amount of subcutaneous

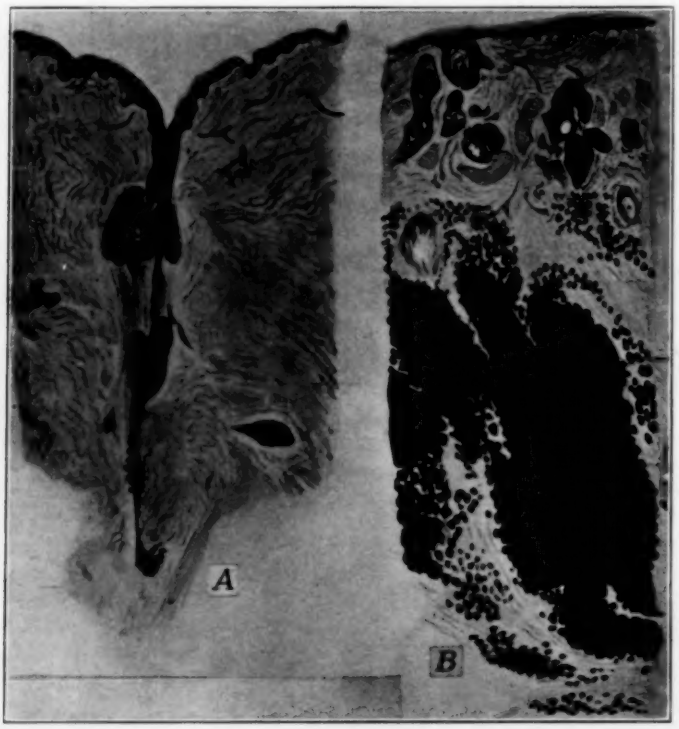


Fig. 3.—Microscopical sections. A. Progressive lipodystrophy. Skin removed to the fascia; almost complete absence of fat; traces in the sebaceous glands and at the roots of the hair. B. Fat content of the normal skin of the head in man 34 years old. Both specimens stained with hematoxylin-Sudan (Simons').

fat gradually increases, but after a certain number of years probably remains stationary, so that, strictly speaking, the disease can no longer be considered progressive. The examination of the parts affected shows no impairment of function of the muscles, no change in the electrical reactions of the muscles or nerves, or in the tendon reflexes, no

disturbances of sensation, no vasomotor disturbances. Roentgenologic examination of the extremities and the skull (sella turcica) shows nothing abnormal. The measurements in the seven cases in which they are given are as shown in Table 1.

TABLE 1.—Measurements of Seven Patients with Lipodystrophy

Author	Age, Years	Weight, Kg.	Height, Cm.	Circumference Arm, Cm.	Forearm, Cm.	Thigh, Cm. *	Thigh, Cm. †	Leg, Cm.
Simons	21	46.8	158	....	....	56	....	....
Simons	28	56.0	159	....	....	61	51.5	37.5
Laignel-Lavastine and Viard	39	53.0	150	22	20	56	46.0	36.0
Cohn	17	....	...	....	....	57	53.5	39.0
Feer	9½	26.9	130	16.5	15.5	..	36.0	26.5
Boissonas	7½	22.1	119	17	18.0	..	34.5	26.5
Herrman	32	55.5	150	22	21.5	62	53.5	37.5

* Measured at the level of the great trochanter.
† Measured 15 cm. above the patella.

The measurements of the thickness of the fold of raised skin and subcutaneous fat,[15] as first suggested by Oeder,[16] and carried out on children by Batkin,[17] are given by only three authors as set forth in Table 2.

TABLE 2.—Measurements of Thickness of Fold of Skin and Subcutaneous Fat

Author	Age, Years	Cheek, Cm.	Breast, Cm.	Axilla, Cm.	Arm, Cm.	Forearm, Cm.	Thigh, Cm.	Leg, Cm.
Laignel-Lavastine	39	..	..	...	...	...	64	59
Feer	9½	4	3	3.5	3.5	2.5	30	18
Herrman	32	4	6	6.0	5.0	5.0	45	27

In none of the patients were changes found in the internal organs which could be attributed to the disease. The examination of the blood

15. The measurement of the thickness of the layer of subcutaneous fat is made as follows: A fold of skin with the subcutaneous fat is raised from the underlying muscle and held by an assistant between the thumb and index fingers of both hands, the thumbs being separated 3 or 4 cm. This double layer of skin and fat is then measured with a pair of calipers; not the kind which are used for measuring the diameters of the head or pelvis, but the kind used by mechanics. This touches the fold along a larger surface and the thickness can be more accurately determined.

16. Oeder: Med. Klin., 1910, p. 17; Fortschr. d. Med., 1911, p. 41.

17. Batkin: Jahrb. f. Kinderh., 1915, lxxxii, 103.

and the urine, and the roentgenologic examination have shown no characteristic changes. In those cases which were tested no disturbance of metabolism and no vasomotor or trophic disturbances were noted. Three hundred grams of grape sugar have been given without producing an alimentary glucosuria; no increase of lipase has been found in the blood; after the ingestion of 200 gm. of a 20 per cent. cream there was no lipemia; the blood serum obtained by puncture of a vein was clear. When large quantities of cream and butter were taken the feces

Fig. 4.—Boissonas' case, a girl aged 10. Disappearance of subcutaneous fat in the face, arms and chest; marked increase in the gluteal region, abdomen, hips and calves.

did not show any marked change. Injections of pilocarpin produced moderate sweating, less distinct on the face; the pulse, respiration and intestinal peristalsis were not affected.

Macroscopically a section of the skin of the affected part shows a marked diminution or a complete absence of fat; microscopically (Simons' Fig. 3) almost a complete loss of the subcutaneous fat; only in the sebaceous glands and at the roots of the hair can traces of fat be seen. The epithelium and the corium present no changes.

Diagnosis and Differential Diagnosis.—If we bear in mind the characteristic features of this condition—bilateral, symmetrical loss of fat in the upper part of the body, with increase of adipose tissue in the lower part, and the absence of other trophic and constitutional disturbances—the diagnosis is usually easy. The cadaverous expression of the face in an otherwise healthy individual is almost pathognomonic. The good general health immediately excludes tuberculosis, malignant

Fig. 5.—Pulmonary tuberculosis in a girl 9 years old (Schlossmann).

tumors and diabetes. The retention of the normal function of the muscles excludes the various forms of muscular atrophy and dystrophy; the normal sexual characteristics and the absence of ocular disturbances separate it from dyspituitarism of the Froelich type.

In passing, it is interesting to note that in this disease the subcutaneous fat is lost in a location in which in infantile atrophy, even when extremely marked, it is retained, namely, as the fat or sucking pad of the cheek. On the other hand, in some cases of advanced pulmonary tuberculosis in children, the fulness of the face may persist after the rest of the body is greatly emaciated (Fig. 5). It is not uncommon

to see girls and young women in whom there is a striking contrast between the thinness of the face and the normal or more than normal development of the lower part of the body; and although I do not believe that such cases should be included in the group of progressive lipodystrophy, they may possibly bear some relation to that condition.

Prognosis.—Although the disappearance of the subcutaneous fat of the face is progressive, after it has all been lost the process must of necessity come to a standstill. A spontaneous reappearance of the fat is extremely unlikely. The increase in adipose tissue in the lower part of the body is also progressive, but after a certain number of years apparently reaches its maximum. The disease appears to interfere in no way with the general health, and it is doubtful if life is shortened.

Treatment.—Thyroid, pituitary, and ovarian extract have been tried without benefit. Feer proposes to use the milk of a thyroidectomized goat, with which he obtained good results in a case of Graves' disease. The rationale of this method of treatment is not clear, since no similar disturbances have been noted in this disease. It would seem more logical to inject the blood of a patient in the active progressive stage of the disease into monkeys with the idea of stimulating the production of antibodies and later to inject the serum of such animals into the patient.

The patients are all female and naturally complain, chiefly, of the disfigurement, especially if they are still unmarried. For cosmetic reasons human fat and mutton suet have been injected into the hollows of the face, but after a time this is absorbed. Transplantation of the excess fat from the buttocks to the face has been suggested, but it is doubtful whether that will remain unabsorbed. Paraffin injections have been objected to because they interfere somewhat with the play of the features; but in view of the great deformity this does not appear a strong objection, and this is the method which I intend to employ in my patient.

250 West Eighty-Eighth Street.

A STUDY OF THE BUFFER VALUE OF THE BLOOD*

R. L. LEVY, M.D., AND L. G. ROWNTREE, M.D.
BALTIMORE MINNEAPOLIS

In order that the various functions of the body may be properly performed, it is essential that the blood maintain its slightly alkaline reaction within extremely narrow limits. When this degree of alkalinity is diminished, a condition of acidosis, with its accompanying phenomena, is observed. A truly acid reaction of the blood (that is, a hydrogen-ion concentration greater than pH-7.0†) is incompatible with life.

Various protective mechanisms serve to maintain the acid-base equilibrium of the organism and thereby protect the blood from significant changes in hydrogen-ion concentration. Such processes are increased production of ammonia, the excretion of carbon dioxid by the lungs, the excretion of nonvolatile acids by the kidneys, and finally, the buffer action of the blood itself.[1]

By the term "buffer action" of a mixture is meant its ability to take up considerable amounts of acid or alkali when these are added to it, without appreciable change in hydrogen-ion concentration. The blood is such a buffer mixture, owing largely to its content of carbonates and phosphates, and, to a lesser extent, its protein.

Much valuable work has been done to determine the rôle played by each of the factors concerned in the maintenance of acid-base equilibrium, but a precise investigation[2] of the "buffer value" of the blood has not been undertaken, owing to technical difficulties. The utilization of the recently-described dialysis-indicator method[3] for determining variations in the hydrogen-ion concentration of the blood has made possible a quantitative study of its buffer value.

---

* Submitted for publication Jan. 20, 1916.

* From the Medical Clinic of the Johns Hopkins Hospital.

† pH is the commonly accepted symbol for hydrogen-ion concentration.

1. For a detailed discussion of the mechanisms involved in the maintenance of acid-base equilibrium in the body, see Henderson, L. J., Ergebn. d. Physiol, 1909, viii, 254.

2. During the course of this study, a preliminary communication on "The Nature and Detection of Diabetic Acidosis," by Van Slyke, Stillman and Cullen, appeared in the Proc. Soc. Exper. Biol. and Med., 1915, xii, 165. The fact that the dialysis-indicator method was being utilized for determining the buffer value of the blood was announced with the presentation of our method before the Association of American Physicians, May, 1915. (See Tr. Assn. Am. Phys., 1915, and abstract in Jour. Am. Med. Assn., 1915, lxiv, 2162.)

3. Levy, R. L., Rowntree, L. G., and Marriott, W. McKim: A Simple Method for Determining Variations in the Hydrogen-Ion Concentration of the Blood, THE ARCHIVES INT. MED., 1915, xvi, 389.

TECHNIC

The determinations may be carried out on whole blood, serum or plasma, but it is preferable to use whole blood, since in this way conditions in the body are most closely approximated.

Two cubic centimeters of blood are placed in each of seven test tubes and allowed to stand for five or six minutes, until a thin layer of plasma at the top has been cleared of cells, hemolysis on the subsequent addition of acid and alkali thereby being prevented. The blood in the first tube is used for a determination of the pH. To each of the next three tubes is added fiftieth-normal hydrochloric acid—0.1 c.c to the first, 0.2 c.c. to the second, and 0.3 c.c to the third. Similarly, increasing amounts of fiftieth-normal sodium hydroxid solution are added to the last three tubes. The tubes are inverted once for the purpose of mixing. Each portion of blood is then separately dialyzed for six minutes against 2.5 c.c. of 0.8 per cent. salt solution and the pH of the dialysate determined by adding 5 drops of an indicator, phenolsulphonephthalein, the reading being determined by comparing with a series of standard colors.

Numerous experiments were carried out to determine the influence of various factors on the results.

1. *Effect of Temperature.*—The dialysate at temperatures ranging from 18 to 37 C. may be identical or may show slight changes,[4] but within these extremes, no essential change is seen in the buffer value of either blood or serum. The determinations can, therefore, be carried out at room temperature, without regard for the slight variations so encountered.

2. *Length of Time Elapsing Between the Withdrawal of Blood and the Carrying Out of Determinations.*—The blood may be kept for as long as twenty-four hours without change in the pH or buffer values, providing it is collected in hard glass tubes which are filled to the top, tightly stoppered, and immediately placed on ice.

3. *Length of Time the Acid or Alkali is in Contact with Blood or Serum.*—A series of experiments was carried out in which the time of contact varied from two to thirty minutes. Within these limits, identical buffer values were obtained in all cases.

4. *Loss of Carbon Dioxid.*—Obviously, the carbon dioxid tension in the blood is important. Control experiments, using the dialysis-indicator method with carbon dioxid at its normal tension, and with blood from which carbon dioxid had been shaken out, showed marked differences in pH. It is possible to collect the blood in such a way that the loss of carbon dioxid is minimal and fairly constant. This is accomplished by withdrawing blood with a syringe or pipet, filling the test tube practically to the top, promptly stoppering, avoiding unnecessary shaking, and placing immediately on ice. Duplicate determinations on the same sample, or on different specimens from the same individual, yield essentially identical results under these conditions.

4. Levy, Rowntree and Marriott, loc. cit., note 3. The time of exposure to a higher temperature is obviously a determining factor.

MODE OF EXPRESSION OF RESULTS

All results are expressed in terms of cubic centimeters of fiftieth-normal hydrochloric acid or sodium hydroxid per 2 c.c. of blood. The following terms are used:

1. *Buffer for Acid or Alkali.*—These represent the amounts of acid or alkali which can be added to blood or serum without change in the pH of the dialysate.[5] The sum of the buffer for acid and that for alkali yields the *total buffer.*

2. *Reserve Buffer for Acid or Alkali.*—These represent the amounts of acid or alkali which can be added to blood or serum without causing a change in the pH of the dialysate beyond the limits of normal.[6] The sum of the two yields the *total reserve buffer.* The reserve buffer, therefore, represents the simple buffer value *plus* the amount of acid or alkali taken care of by the blood without change in reaction beyond the limits of normal pH values. From a clinical standpoint it is the simple buffer values for acid and alkali which are of the greatest significance.

ANALYSES OF CLINICAL RESULTS

The work is based on a study of sixty-five cases, involving considerably more than 100 buffer determinations. The results may best be presented by dividing the cases into four groups.

1. *Normal Individuals (Twenty-Four Cases).*[7]—Determinations were made on both blood[8] and serum, the results appearing in Tables 1 and 2. It is noteworthy that the time elapsing between the previous meal and the withdrawal of blood for examination made no essential difference in the buffer values.

(*a*) *Blood.*—Normal blood takes 0.1 c.c. of acid or alkali without change in pH. It, therefore, has a buffer for acid and alkali of at least 0.1, though it may have a buffer of 0.2 to 0.4. The average buffer, both for acid and alkali is 0.18. The total buffer ranges from 0.2 to 0.7, with a group average of 0.36.

The reserve buffer[9] for both acid and alkali varies from 0.1 to 0.4, the group average for the acid being 0.27, that for the alkali 0.31. The total reserve buffer varies from 0.4 to 0.7, the group average being 0.58.

(*b*) *Serum.*[10]—Normal serum always takes 0.1 c.c. of acid without change in pH. It may have a buffer for acid of from 0.1 to 0.3; the

5. No attempt was made to determine changes smaller than 0.05.

6. Normal pH values are: Blood, 7.4 to 7.6; Serum, 7.6 to 7.8.

7. The blood on which these determinations were made was obtained from patients in the Genito-Urinary Dispensary, a majority of whom were under treatment for local infections of the lower genito-urinary tract. No luetic individuals are included in the series.

8. The blood was collected in tubes containing just sufficient carbonate-free sodium oxalate to prevent clotting.

9. The reserve values throughout are not absolute, since in many instances 0.3 c.c of acid and alkali failed to carry the pH beyond normal limits.

10. The values for oxalated plasma are essentially the same as those for serum. Throughout the later studies only whole blood was utilized, for the following reasons: 1. In the body it is with the buffer of the blood as a whole with which we are concerned. 2. Less blood suffices. 3. Centrifugalization, together with the resultant loss of carbon dioxid, is avoided.

TABLE 1.—Normal Cases

Case No.	Blood									Serum										Time Elapsing Since Last Meal, Hours
	pH	+ N/50 HCl (c.c.)				+ N/50 NaOH (c.c.)				pH	+ N/50 HCl (c.c.)				+ N/50 NaOH (c.c.)					
		0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4		0.1	0.2	0.3	0.4	0.05	0.1	0.2	0.3	0.4	
1	7.6	7.6	7.6	7.55	7.5	7.6	7.7	7.8	7.8	7.8	7.8	7.7	7.6	7.5	....	7.8	7.95	7.95	8.2	1¾
2	7.55	7.55	7.55	7.45	7.45	7.55	7.55	7.6	7.65	7.7	7.7	7.7	7.6	7.5	....	7.7	7.9	8.0	8.0	2
3	7.6	7.6	7.6	7.5	7.45	7.6	7.6	1.65	7.7	7.7	7.7	7.65	7.55	....	....	7.7	7.8	7.8	7.9	
4	7.5	7.5	7.5	7.35	7.3	7.5	7.55	7.6	....	7.7	7.7	7.7	7.65	7.6	....	7.7	7.8	7.95	7.95	6
5	7.55	7.55	7.55	7.45	7.45	7.55	7.55	7.65	7.7	7.7	7.7	7.65	7.6	7.55	....	7.9	7.95	8.0	8.05	2
6	7.55	7.55	7.45	7.35	7.3	7.55	7.55	7.55	7.55	7.7	7.7	7.7	7.55	7.55	....	7.8	7.9	7.95	8.0	2
7	7.45	7.45	7.45	7.45	7.3	7.45	7.45	7.55	7.55	7.7	7.7	7.65	7.6	7.5	....	7.7	7.95	8.0	8.1	1¾
8	7.55	7.45	7.45	7.35	7.3	7.55	7.6	7.6	7.6	7.7	7.7	7.6	7.5	7.45	....	7.7	7.9	7.95	8.0	4½
9	7.55	7.55	7.5	7.45	7.45	7.55	7.55	7.65	7.65	7.7	7.7	7.7	7.6	7.5	7.75	7.9	7.9	8.0	8.0	2½
10	7.45	7.45	7.45	7.3	7.25	7.45	7.55	7.55	7.55	7.7	7.7	7.55	7.5	7.45	....	7.7	7.9	7.95	8.0	2
11	7.45	7.45	7.3	7.25	7.25	7.45	7.45	7.55	7.55	7.7	7.7	7.55	7.45	7.45	....	7.7	7.9	7.9	7.9	2½
12	7.45	7.45	7.35	....	7.35	7.45	7.45	7.5	7.5	7.65	7.65	7.55	7.45	7.45	7.65	7.75	7.95	8.0	..	1¾
13	7.45	7.45	7.45	7.45	7.35	7.45	7.45	7.45	7.45	7.65	7.65	7.55	7.45	7.35	7.65	7.75	7.85	8.05	..	2
14	7.45	7.45	7.45	7.35	7.35	7.45	7.5	7.55	7.55	7.65	7.65	7.55	7.45	7.35	7.65	7.85	7.85	8.05	..	2
15	7.45	7.45	7.45	7.35	7.35	7.45	7.45	7.5	7.55	7.65	7.65	7.45	7.45	7.45	7.65	7.8	7.8	7.85	..	21
16	7.45	7.45	7.45	7.4	7.35	7.45	7.55	7.55	7.55	7.8	7.8	7.6	7.55	7.45	7.8	7.8	7.8	7.9	..	5
17	7.45	7.45	7.45	7.4	7.4	7.45	7.55	7.7	7.75	7.7	7.7	7.65	7.6	7.55	7.7	7.8	7.9	8.05	..	2
18	7.45	7.45	7.45	7.4	7.35	7.45	7.55	7.65	7.7	7.65	7.65	7.45	7.45	7.35	7.65	7.8	7.9	7.9	..	3
19	7.45	7.45	7.35	7.35	7.35	7.45	7.55	....	7.55	7.75	7.75	7.7	7.6	7.5	7.85	7.9	7.9	8.1	..	2
20	7.55	7.55	7.45	7.45	7.45	7.55	7.55	7.7	....	7.75	7.75	7.65	7.65	7.55	7.75	7.75	7.9	8.1	..	2
21	7.55	7.55	7.55	7.55	....	7.55	7.55	7.55	....	7.65	7.65	7.55	7.45	....	7.65	7.75	7.75	7.8	..	2
22	7.45	7.45	7.45	7.45	....	7.45	7.45	7.5	....	7.65	7.65	7.6	7.5	....	7.65	7.75	7.85			
23	7.45	7.45	7.45	7.45	....	7.45	....	7.55	....	7.65	7.65	7.55	7.55	....	7.65	7.65	7.75			
24	7.45	7.45	7.35	7.35	....	7.45	7.45	7.45	....	7.65	7.65	7.55	7.45	....	7.65	7.7	7.8			

average is 0.11. Its buffer for alkali is very variable; a change may be encountered on the addition of from 0.05 to 0.3 c.c. of fiftieth-normal sodium hydroxid; the average is 0.07. The total buffer for serum varies from 0.1 to 0.3, the group average being 0.18.

The reserve buffer for acid varies from 0.1 to 0.4, the group average being 0.2. The reserve buffer for alkali ranges from 0 to 0.3, the group average being 0.12. The total reserve buffer varies from 0.15 to 0.6, the average being 0.33.

TABLE 2.—Summary of Twenty-Four Normal Cases

		Blood		Serum	
		Extremes	Group Average	Extremes	Group Average
Buffer	For acid	0.2 - 0.4	0.18	0.1 - 0.3	0.11
	For alkali	0.2 - 0.4	0.18	0 - 0.3	0.07
	Total	0.2 - 0.7	0.36	0.1 - 0.3	0.18
Reserve buffer	For acid	0.2 - 0.4	0.27	0.1 - 0.4	0.2
	For alkali	0.2 - 0.4	0.31	0 - 0.3	0.12
	Total	0.4 - 0.7	0.58	0.15 - 0.6	0.33

TABLE 3.—Miscellaneous Cases with Normal pH and Normal Buffer Values; Sixteen Cases; Twenty Determinations

		Blood	
		Extremes	Group Average
Buffer	For acid	0.1 - 0.3	0.13
	For alkali	0.1 - 0.3	0.17
	Total	0.2 - 0.5	0.3
Reserve buffer	For acid	0.1 - 0.3	0.25
	For alkali	0 - 0.3	0.23
	Total	0.3 - 0.6	0.48

Although the total buffer value or the total reserve value may be great, the buffer for either acid or alkali may be small; in other words, the buffer may be great for acid and small for alkali, or vice versa. This can be determined only by actual study of the individual acid and alkali buffer values, and it is obviously not shown by a figure expressing the total buffer value.

2. *Miscellaneous Cases with Normal pH and Normal Buffer Values.*—Twenty determinations were made on sixteen cases, including instances of diabetes mellitus, pernicious anemia, myeloid leukemia, typhoid fever, pregnancy, surgical cases before and after anesthesia, exophthalmic goiter and chronic nephritis (Table 3).

TABLE 4.—Cases with Normal pH Showing Diminished Buffer Values; Sixteen Cases; Twenty-Five Determinations

Case No.	Medical No.	Diagnosis	Date	Blood							Remarks*
				pH	+ N/50 HCl (c.c.)			+ N/50 NaOH (c.c.)			
					0.1	0.2	0.3	0.1	0.2	0.3	
1	23988	Intestinal parasitism; (Balantidium coli); marked anemia	4/22/15	7.45	7.45	7.45	7.45	7.7	7.75	7.75	Alveolar CO_2 tension, 28.6 mm.; blood—R. B. C., 1,548,000; Hb., 20 per cent. (Sahli).
2	34083	Bronchopneumonia..	4/23/15	7.4	7.3	7.3	7.25	7.45	7.5	7.5	Alveolar CO_2 tension, 16.2 mm.; extreme prostration with mental torpor; uremia suspected; died; necropsy showed no renal lesions.
3	34093	Chronic nephritis; hypertension; albuminuric retinitis	9/10/15	7.45	7.45	7.45	7.45	7.65	7.65	7.65	Blood urea, 102 mg.; phthalein test, 5 per cent. in two hours; Ambard's K., 0.715.
			10/ 7/15	7.55	7.55	7.55	7.55	7.55	7.7	7.75	Blood urea, 78 mg.; Ambard's K., 0.572.
4	34753	Mercuric chlorid poisoning	9/24/15	7.6	7.55	7.55	7.45	7.6	7.65	7.65	Alveolar CO_2 tension September 25, 26.8 mm.; fifth day since taking 7½ grain tablet; anuric since.
			9/27/15	7.55	7.55	7.55	7.55	7.65	7.75	7.75	Eighth day, voided 110 c.c.; anuria for seven days preceding; intensive alkali therapy.
			9/28/15	7.7	7.7	7.65	7.45	7.7	7.75	7.85	Alveolar CO_2 tension, 33 mm.; 485 c.c. urine voided; blood T. N. P. N., 173 mg.
			9/29/15	7.65	7.65	7.65	7.55	7.65	7.75	7.8	Phthalein, 0; Ambard's K., 1.09; 1,185 c.c. urine voided.
			10/ 6/15	7.5	7.5	7.5	7.45	7.6	7.65	7.65	Alveolar CO_2 tension, 30.6 mm.; 1,770 c.c. urine voided; T. N. P. N. blood, 42 mg.; phthalein, 27 per cent. in two hours; Ambard's K., 0.186.
			10/ 8/15	7.55	7.55	7.45	7.45	7.55	7.6	7.65	Alveolar CO_2 tension, 39.1 mm.; 2,120 c.c. urine voided; T. N. P. N. blood, 26 mg.; phthalein, 35 per cent. in two hours; Ambard's K., 0.124; recovered.
5	34085	Diabetes mellitus; chronic nephritis	9/24/15	7.45	7.4	7.35	7.35	7.55	7.55	7.55	Alveolar CO_2 tension September 26, 22.8 mm.; voluntary starvation; much acetone and diacetic acid in urine; getting sod. bicarb., 2 dr. every four hours.
6	34774	Diabetes mellitus (renal)	9/30/15	7.6	7.5	7.5	7.45	7.6	7.6	7.6	Before starvation, blood sugar, 0.13 per cent.
			10/ 4/15	7.45	7.45	7.45	7.45	7.45	7.45	7.45	Starved for past four days; no alkali; Ambard's K., 0.112; blood urea, 29 mg.
			10/ 7/15	7.45	7.45	7.45	7.4	7.45	7.45	7.6	Starved for seven days; no alkali; persistent glucosuria without hyperglycemia; acetone and diacetic acid in urine.
7	34776	Cerebral arteriosclerosis; cerebral hemorrhage	9/30/15	7.45	7.45	7.45	7.45	7.5	7.55	7.55	In coma; marked cyanosis; respiration labored; died same day.
8	34831	Typhoid fever.....	10/14/15	7.5	7.5	7.5	7.45	7.6	7.6	7.6	Temperature at 2 p. m., 103 F.; ice sponge at 2:15; blood drawn at 2:40; patient having chill.
9	34845	Typhoid fever.....	10/14/15	7.45	7.45	7.45	7.45	7.55	7.6	7.65	Temperature at 2 p. m., 103 F.; ice sponge at 2:15; blood drawn at 3.
10	34869	Diabetes mellitus.....	10/26/15	7.55	7.55	7.45	7.45	7.65	7.65	7.65	Acetone and diacetic acid in urine; has been getting 24 gm. sod. bicarb. daily for past six days; alveolar CO_2 tension, 32.1 mm.; blood sugar, 0.138 per cent.
11	34932	Acute tuberculous pneumonia	10/27/15	7.45	7.45	7.45	7.45	7.5	7.65	7.7	Consolidation of entire right lung and part of left lung; respirations labored, rapid and shallow.
12	34944	Bacillary dysentery..	10/28/15	7.55	7.55	7.5	7.45	7.6	7.6	7.65	Prolonged diarrhea; 10 to 12 stools daily for several weeks; marked emaciation and weakness; died.
13	G. U.	Hypertrophy of prostate; operation	11/ 3/15	7.45	7.45	7.45	7.4	7.45	7.55	7.55	Just before operation (control).
				7.4	7.35	7.35	7.3	7.4	7.4	7.4	Immediately after operation; perineal prostatectomy; gas and ether (1 fl. oz.) anesthesia lasting 40 minutes.
14	Obst.	Pregnancy (9th mo.)	11/ 4/15	7.45	7.45	7.45	7.45	7.5	7.55	7.6	
15	Obst.	Pregnancy (9th mo.)	11/ 4/15	7.45	7.45	7.45	7.45	7.55	7.55	7.55	
16	Bay View	Acute yellow atrophy of liver	11/17/15	7.45	7.45	7.45	....	7.55	7.55	....	Alveolar CO_2 tension, 34.1 mm. Hg; in coma; died three days later; necropsy.

* The values of Ambard's coefficient, total N. P. N. and urea were determined by the staff of the Chemical Division of the Medical Clinic, to whom we are indebted for the privilege of using them. The nitrogen and urea figures represent mg. per 100 c.c. We desire to thank Dr. J. H. King for a number of alveolar CO_2 determinations.

The values obtained are uniformly slightly lower than those observed in the series of normals, but otherwise present no features of especial interest.

3. *Cases with Normal pH and Diminished Buffer Values.*—(Tables 4 and 5.) An analysis of these tables shows essentially normal buffer and reserve buffer values for acid, markedly diminished values for alkali, and therefore lowered values both for total and total reserve buffers.

Several points of interest are evident from a consideration of this group of cases. It is striking that a diminished buffer for alkali is far more common than that for acid in cases which do not show a *true* acidosis, but in which there is a tendency toward acidosis, as evidenced in many instances by a lowering of the tension of the alveolar carbon

TABLE 5.—Cases with Normal pH and Diminished Buffer Values; Sixteen Cases; Twenty-Five Determinations

		Blood	
		Extremes	Group Average
Buffer	For acid	0 - 0.3	0.19
	For alkali	0 - 0.3	0.06
	Total	0 - 0.6	0.25
Reserve buffer	For acid	0 - 0.3	0.26
	For alkali	0 - 0.3	0.18
	Total	0.3 - 0.6	0.44

dioxid. (Cases 1, 2, 4, 5, 10 and 16.) The diminished buffer for alkali observed in two cases of normal pregnancy (Cases 14 and 15) is in accordance with the almost constant finding of a lowered alveolar carbon dioxid tension during the months of gestation.[11]

Case 4 (mercuric chlorid poisoning) shows at various stages during the clinical course several phases of buffer loss. At first, after five days of anuria, there was loss of buffer for acid; with slight clinical improvement and the reestablishment of urinary secretion, buffer for acid returned, whereas that for alkali was diminished. After intensive alkali therapy, an alkalosis was established though the alveolar carbon dioxid was still somewhat lowered; the buffer values were normal. October 6, a slight set-back occurred, with loss of buffer for alkali. It was prognosticated at this time from the buffer determinations that the alveolar carbon dioxid tension, which two days previously had been normal,

11. Leimdörfer, A., Novak, J., and Porges, O.: Ztsch. f. klin. Med., 1912, lxxv, 301.

we are indebted for the privilege of using them. The nitrogen and urea figures represent mg. per 100 c.c. We desire to thank Dr. J. H. King for a number of alveolar CO_2 determinations.

TABLE 6.—Acidosis; Nine Cases; Twenty-Four Determinations

Case No.	Medical No.	Diagnosis	Date	Blood							Alveolar CO_2 Tension mm. Hg	Remarks
				pH	$+\frac{N}{50}$ HCl (c.c.)			$+\frac{N}{50}$ NaOH (c.c.)				
					0.1	0.2	0.3	0.1	0.2	0.3		
1	33948	Acute and chronic nephritis; hypertension; uremia; albuminuric retinitis	4/20/15	7.35	7.35	7.35	7.3	7.6	7.6	7.65	....	Patient comatose; Ambard's K., 3.3.
			4/21/15	7.4	7.3	7.2	....	7.4	7.45	....	10.4	Died two days later; necropsy.
2	33799	Chronic nephritis; uremia	4/20/15	7.2	7.15	7.15	7.1	7.25	7.45	7.45	....	Ambard's K., 1.90; T. N. P. N. blood, 192 mg.
			4/21/15	7.15	6.9	6.9	....	7.15	7.25	....	11.0	Dull; nausea and vomiting; headache; died four days later; necropsy.
3	34066	Chronic nephritis; hydronephrosis (left); uremia; secondary anemia	4/21/15	7.05	7.0	6.9	....	7.15	7.2	....	0.0	Patient comatose; marked "air hunger"; two hours later, given 500 c.c. blood by syringe transfusion and 400 c.c. 4 per cent. sod. bicarb. intravenously; symptomatically unimproved, but at end of injection, pH of blood = 7.45; died with acute pulmonary edema four hours after the transfusion; Ambard's K. = 3.68 ten hours before death; T. N. P. N. blood, 232 mg.
4	34725	Chronic nephritis; hypertension; uremia; secondary anemia	10/ 4/15	7.35	7.35	7.3	7.15	7.35	7.4	7.45	25.0	T. N. P. N. blood, 181 mg.
			10/ 5/15	7.35	7.25	7.15	7.15	7.35	7.45	7.45	15.7	Venesection for 650 c.c. blood on October 4.
			10/ 7/15	7.35	7.3	7.0	6.9	7.45	7.45	7.55	11.0	Given 300 c.c. 4 per cent. sol. sod. bicarb. + 200 c.c. 5 per cent. glucose sol. intravenously on this day; alveolar CO_2 tension immediately before injection, 11 mm.; immediately after, 17.5 mm.; marked "air hunger"; respirations, 8 per minute; vomiting.
			10/ 8/15	1.35	7.25	7.15	7.15	7.45	7.45	7.45	....	Twenty-four hours later; at this time given 400 c.c. of 4 per cent. sol. sod. bicarb.
				7.55	7.55	7.45	7.35	7.55	7.6	7.7	....	Immediately after injection of the sod. bicarb.; respirations somewhat faster; still in coma.
				7.55	7.45	7.4	7.35	7.55	7.6	7.6	....	One and one half hours after alkali injection; breathing slow and labored; respirations, 8 per minute.

			10/ 9/15	7.35	7.3	7.25	7.2	7.45	7.45	7.55	....	Twenty-four hours later; marked "air hunger"; respirations, 12 per minute; in deep coma; at this time, given 300 c.c. of 4 per cent. sol. sod. bicarb. + 300 c.c. of a phosphate mixture (KH_2PO_4 + Na_2HPO_4) having pH of 7.4.
				7.55	7.5	7.45	7.4	7.55	7.6	7.7	....	Immediately after the injection; no change in condition; died two hours later; urine alkaline throughout stay in hospital; pH 7.6.
5	G. U.	Ureteral kink; hydronephrosis; operation	10/17/15	7.45	7.45	7.45	7.4	7.45	7.55	7.55	....	Just before operation. (Control.) Patient in excellent condition.
				7.35	7.35	7.2	7.2	7.35	7.35	7.4	....	Immediately after operation; cutting of aberrant vessels pressing on ureter; gas anesthesia lasting 1 hour and 35 minutes; uneventful postoperative recovery.
6	34966	Diabetes mellitus; syphilis; aneurism of thoracic aorta	11/ 4/15	7.15	7.15	7.15	7.15	7.15	7.2	7.2	30.0	Comatose; marked "air hunger"; blood sugar, 0.6 per cent.
			11/ 5/15	7.45	7.35	7.35	7.25	7.45	7.45	7.45	28.0	Conscious, though mentally dull; during past twenty-four hours received about 1 oz. of sod. bicarb. by mouth, and 500 c.c. of a saturated solution per rectum; died in coma two days later.
7	35075	Acute and chronic nephritis; uremia	11/24/15	7.3	7.3	7.2	7.2	7.3	7.35	7.35	....	In coma, having repeated convulsions; phthalein, 12 per cent. in two hours.
			11/27/15	7.45	7.45	7.45	7.45	7.65	7.65	7.65	22.4	Mentally clear; much improved after sweating and bleeding.
8	35092	Diabetes mellitus; hydronephrosis; chronic morphinism	11/29/15	7.3	7.3	7.15	7.15	7.35	7.35	7.35	23.3	Drowsy, though mentally clear; respirations rather deep, no "air hunger"; blood urea, 16 mg.; phthalein test, 42 per cent. in two hours; blood sugar, 0.33 per cent.
			12/ 5/15	7.35	7.3	7.15	7.15	7.35	7.35	7.35	19.5	Alveolar CO_2 November 30 = 35.0 mm.; starved; blood sugar, 0.25 per cent.
			12/ 9/15	7.45	7.45	7.45	7.4	7.45	7.5	7.5	23.0	After intensive intravenous alkali therapy; still mentally dull; getting small amount of broth, green vegetables, and eggs; died two days later.
9	35034	Carcinoma of stomach; large hepatic metastases; marked secondary anemia	12/10/15	7.35	7.35	7.15	7.15	7.35	7.35	7.45	12.1	Marked "air hunger"; stuporous; R. B. C., 1,800,000; Hb. (Sahli), 23 per cent.; phthalein, 53 per cent. in two hours.
			12/11/15	7.35	7.35	7.35	7.15	7.45	7.45	7.55	11.4	Condition unchanged; died next day; necropsy.

would be found to be again lowered, which, indeed, proved to be true. Finally, during convalescence, both pH and buffer values became normal..

The loss of buffer for alkali observed in two instances of febrile typhoid (Cases 8 and 9) is in accord with the tendency toward an alkalosis which we have observed in several cases of this disease.

TABLE 7.—Acidosis; Nine Cases; Twenty-Four Determinations

	Blood					
	Acid		Alkali		Total	
	Extremes	Averages	Extremes	Averages	Extremes	Averages
Entire group (9 cases; 24 determinations).....	0-0.3	0.083	0-0.3	0.087	0 -0.4	0.17
During stage of true acidosis (9 cases; 16 determinations).....	0-0.3	0.075	0-0.3	0.056	0 -0.4	0.13
During stage of compensation (6 cases; 8 determinations).....	0-0.3	0.1	0-0.3	0.1	0.1-0.3	0.2

TABLE 8.—Summary of Acid, Alkali and Total Buffer Values for the Various Case Groups Expressed as Group Averages

	Blood		
	Acid	Alkali	Total
Normal individuals	0.18	0.18	0.36
Miscellaneous cases with normal pH, showing normal buffer values.....	0.13	0.17	0.30
Cases with normal pH showing diminished buffer values.....	0.19	0.06	0.25
Acidosis..... True.....	0.075	0.056	0.13
Acidosis..... Compensated.....	0.10	0.10	0.20

4. *Acidosis.*—(Tables 6 and 7.) From the tables it is evident that cases of acidosis have less buffer for both acid and alkali during the stage of uncompensated acidosis (increased pH of the blood) than during the stage in which the tension of alveolar carbon dioxid is diminished, but the pH of the blood is normal (stage of compensation). Despite the return to normal of pH and buffer values, the clinical evidences of acidosis may persist (Cases 4 and 8).

EXPERIMENTAL CONSIDERATIONS

Inasmuch as the buffer value of blood is fairly constant in health, and decreased in acidosis, the question obviously arises as to whether buffer can be supplied. The possibility of accomplishing this with

phosphate mixtures was considered. The buffer values of phosphate mixtures ($Na_2HPO_4 + KH_2PO_4$) of various strengths and hydrogen-ion concentrations, were therefore determined, the results appearing in Table 9.

From this study four interesting facts are evident: First, the buffer value of blood is greater than that of $\frac{1}{15}$ molecular phosphate mixtures and approximately that of $\frac{1}{5}$ molecular mixtures; second, the buffer value is greater at neutrality than in more alkaline mixtures; third, the dialysates exhibit buffer values comparable to those obtained by adding the indicator directly to the original mixtures, but at a

TABLE 9.—Buffer Value of Phosphate Mixtures ($Na_2HPO_4 + KH_2PO_4$)

	1/30 Molecular		1/15 Molecular	1/5 Molecular	
	Undialyzed	Dialysate	Undialyzed	Undialyzed	Dialysate
pH	7.55	7.4	7.3	7.5	7.45
+0.1 acid	7.45	7.35	7.2	7.5	7.45
+0.2 acid	7.4	7.25	7.15	7.45	
+0.3 acid	7.3	7.15	7.15	7.45	
+0.4 acid	7.2	7.1	....	7.4	7.35
+0.1 alkali	7.6	7.5	7.3	7.5	7.45
+0.2 alkali	7.75	7.65	7.35	7.55	
+0.3 alkali	7.8	7.75	7.4	7.55	
+0.4 alkali	8.0	7.8	....	7.6	7.6

ONE-FIFTEENTH MOLECULAR. ALL READINGS ON UNDIALYZED MIXTURES				
pH	7.05	7.3	7.9	8.1
+0.1 acid	7.05	7.2	7.85	7.95
+0.2 acid	7.0	7.15	7.75	7.85
+0.3 acid	6.95	7.15	7.7	7.75
+0.1 alkali	7.05	7.3	7.95	
+0.2 alkali	7.05	7.35	8.0	
+0.3 alkali	7.1	7.4	8.1	

uniformly more acid level; and, finally, the more concentrated the mixture employed, the less marked is the discrepancy between the undialyzed mixture and dialysate.

Attempts to increase the buffer value of the blood by intravenous injection of phosphate mixtures into animals and into a human case (Table 6, Case 4) failed, but demonstrated the following facts:

1. The phoshpate mixtures are relatively nontoxic.[12]

12. See, also, Greenwald, I., Jour. Pharmacol. and Exper. Therap., 1915, vii, 57.

TABLE 10.—Protocol of Experiment 1. Injection into a Dog of Third Normal Hydrochloric Acid, Followed by a 4 Per Cent. Solution of Sodium Bicarbonate*

MALE; WEIGHT, 8.6 KG.

Time	Total Amount Injected (c.c.)		Blood							Alveolar CO_2 Tension, mm. Hg	Remarks
			pH	+ N/50 HCl (c.c.)			+ N/50 NaOH (c.c.)				
	N/3 HCl	4% $NaHCO_3$		0.1	0.2	0.3	0.1	0.2	0.3		
12:10	...	...	7.55	7.55	7.45	7.45	7.55	7.55	7.55	38.6	Dog on table; respirations, 28 per min.; pulse, 31 to ¼.
12:25	⟶	...	....	....	....	....	....	....	....	....	Injection of HCl begun.
12:40	75	...	7.35	7.35	7.35	7.3	7.35	7.35	7.35	31.9	
12:46	150	...	....	....	....	....	....	....	....	....	Breathing is slow and deep; salivating profusely; muscles relaxed; defecating involuntarily; seems much distressed; injection stopped.
12:50	...	...	7.15	7.15	7.1	....	7.15	7.2	7.2		
1:00	155	...	....	....	....	....	....	....	....	....	Marked "air hunger"; involuntary defecation continues.
1:05	...	...	....	....	....	....	....	....	....	27.4	Respirations, 16 per min., deep and irregular; pulse, 23 to ¼.
1:07	165 ⟵	...	....	....	....	....	....	....	....	....	The injection of the last 10 c.c. of acid immediately caused deepening and quickening of respiration.
1:08	...	⟶	....	....	....	....	....	....	....	....	Injection of $NaHCO_3$ begun.
1:14	...	85	....	....	....	....	....	....	....	....	Respirations decidedly shallower; animal still relaxed and quiet.
1:20	...	...	7.35	7.35	7.3	7.2	7.4	7.45	7.45		
1:25	...	120	....	....	....	....	....	....	....	39.5	
1:35	..	250	7.6	7.6	7.5	....	7.6	7.6	7.6	43.8	Animal more animated; struggling.
1:40	..	350	....	....	....	....	....	....	....	....	Free diuresis; pH of urine, 7.65.
1:50	...	500	....	....	....	....	....	....	....	....	Injection stopped.
2:05	...	...	7.7	7.7	7.6	7.6	7.7	7.8	7.8	46.0	
2:12	...	...	....	....	....	....	....	....	....	....	Animal seems weak; breathing quietly; sacrificed.

* Injections made into leg vein; blood for examination withdrawn from jugulars; N/3 HCl made up in 0.8 per cent. salt solution; bicarbonate solution made up in distilled water. Arrows mark beginning and end of injections.

TABLE 11.—Protocol of Experiment 2. Injection into a Dog of a 4 Per Cent. Solution of Sodium Bicarbonate, Followed by N/3 Hydrochloric Acid.* Male; Weight, 6.25 kg.

Time	Total Amount Injected (c.c.)		Blood							Alveolar CO_2 Tension, mm. Hg	Remarks
			pH	$+\frac{N}{50}$ HCl (c.c.)			$+\frac{N}{50}$ NaOH (c.c.)				
	4% $NaHCO_3$	N/3 HCl		0.1	0.2	0.3	0.1	0.2	0.3		
10:50	...	...	7.35	7.35	7.3	7.2	7.35	7.4	7.45	44.4	Dog on table.
10:53	⟶	...	....	....	....	....	....	....	....	....	Injection of alkali begun.
11:15	175	...	....	....	....	....	....	....	....	....	Animal very restless.
11:20	200	...	7.8	7.7	7.7	....	7.8	7.85	....	53.7	Injection stopped.
11:30	...	...	....	....	....	....	....	....	....	....	Injection resumed; quieter.
11:35	275	...	....	....	....	....	....	....	....	....	Deep, snorting respirations; watery defecation.
11:38	355 ⟵	...	....	....	....	....	....	....	....	....	Still defecating at intervals; profuse diuresis; pH urine, 7.85; injection of alkali stopped.
11:50	...	...	7.75	7.7	7.65	7.6	7.75	7.9	7.9	63.7	
12:11	...	⟶	....	....	....	....	....	....	....	....	Injection of acid begun.
12:16	...	50	....	....	....	....	....	....	....	....	Restless.
12:20	...	...	....	....	....	....	....	....	....	....	Respirations 20 per minute, slow, deep and regular.
12:24	...	150	7.35	7.35	7.2	7.15	7.4	7.4	7.5	40.1	
12:38	...	200	....	....	....	....	....	....	....	....	Respirations very slow and deep.
12:42	...	250 ⟵	7.3	7.2	7.15	....	7.3	7.35	7.35	27.9	
1:00	...	...	....	....	....	....	....	....	....	....	Dog seems in fairly good condition. Still passing watery stools. Recovered.

* Injections made into leg vein; blood for examination withdrawn from jugulars; solution of bicarbonate made up in distilled water; HCl made up in 0.8 per cent. salt solution. Arrows mark beginning and end of injections.

2. The injection of sodium phosphate (Na_2HPO_4) (800 c.c. of a ⅕ molecular solution representing 28.5 gm. of the salt) into an animal of 11 kg. did not produce an alkalosis or increase the buffer values, whereas the injection of 750 c.c. of a $\frac{1}{15}$ molecular mixture of di-sodium phosphate (Na_2HPO_4) and acid potassium phosphate (KH_2PO_4) at pH 7.0 into a dog weighing 6.65 kg., produced a mild acidosis without appreciable change in buffer values.

3. The intravenous injection of 300 c.c. of a phosphate mixture at pH 7.4, together with 300 c.c. of a 4 per cent. solution of sodium bicarbonate in the case of a man dying in uremic coma, caused a change in the pH of the blood from 7.35 to 7.55, and increased the buffer for alkali but not for acid. There was no change in the clinical condition of the patient, who died two hours later.

Buffer Values in Experimental Acidosis and Alkalosis.—(Tables 10 and 11). It is seen from Experiment 1 that in the acidosis produced by the intravenous injection of an inorganic acid (hydrochloric acid) the buffer values may remain practically normal, although there is a decided lowering of the alveolar carbon dioxid and a marked increase in the pH of the blood. This is in accord with what is sometimes found in the acidosis of chronic nephritis with uremia and in that of diabetes (Table 6, Cases 4 to 9, inclusive). After injection of alkali to overcome the acidosis, there is a transient diminution in the buffer for alkali, followed by a prompt return to normal values as the pH of the blood is lowered.

In experimental alkalosis the buffer for acid is first diminished. The alveolar carbon dioxid rises to unusually high figures—63.7 mm. in this animal. After injection of sufficient hydrochloric acid to overcome the alkalosis and produce a mild acidosis with lowered alveolar carbon dioxid tension, the buffer for both acid and alkali is lowered.

In the instance of mercuric chlorid poisoning (Case 4, Table 4), in which the patient received large doses of sodium bicarbonate both by mouth and intravenously, a similar condition of alkalosis was produced, though here the buffer values remained normal at this stage of the disease, and the alveolar carbon dioxid tension was still low—33.0 mm. of mercury.

SUMMARY

1. A simple method is described for determining quantitatively the buffer value of the blood. It consists in adding increasing amounts of fiftieth-normal hydrochloric acid and fiftieth-normal sodium hydroxid solution to equal quantities of blood and observing the resulting changes in hydrogen-ion concentration by means of the dialysis-indicator method.

2. The results are expressed in terms of cubic centimeters of fiftieth normal acid or alkali per 2 c.c. of blood.

3. The buffer values of blood for acid and alkali yield valuable information from a clinical standpoint. The reserve and total buffers may also be calculated from the results of the determinations.

4. The minimal values for normal blood have been determined; considerably larger values may be encountered. For a group of miscellaneous cases with normal pH, the average buffer values were somewhat lower, though within normal limits.

5. In certain cases with normal pH of the blood, but showing a tendency toward the development of an acidosis (as evidenced, for example, by lowered alveolar carbon dioxid tension), the buffer values were diminished. The loss of buffer for alkali was far more striking and frequent than for acid, and was often associated with lowered alveolar carbon dioxid tension. As a result of therapy, especially the use of alkali, the buffer values in some instances returned to normal.

6. In a series of cases of acidosis the average buffer values were found to be markedly diminished, particularly during the stage in which the pH of the blood was abnormally high. Normal buffer values may be encountered, however, in the presence of a true acidosis. Coincident with clinical improvement following treatment, particularly intensive alkali therapy, both pH and buffer values approximated or became normal in several cases.

7. It was not possible to supply buffer to the blood by the injection of phosphate mixtures.

8. By the intravenous injection of third-normal hydrochloric acid and 4 per cent. sodium bicarbonate solution into dogs, conditions of acidosis and alkalosis were produced which were inconstantly accompanied by changes in the buffer values of the blood.

9. The determination of the buffer value of the blood, especially in cases in which an acidosis is suspected or present, yields information of some diagnostic and prognostic significance and permits of a more complete study of the factors concerned in the maintenance of the acid-base equilibrium of the body.

AN ADAPTATION OF THE ERLANGER CAPSULE TO ANY POLYGRAPH FOR OBTAINING ARTERIAL PULSE RECORDS *

ROBERT H. HALSEY, M.D.
NEW YORK

It is now generally recognized that to understand properly the action of the heart it is necessary to employ mechanical instruments which register for comparison the simultaneous events in its various chambers.

For the recording of events in the cardiac cycle two types of instruments have been devised: one, for recording the ventricular contraction, depends on the application to the artery at the wrist of a metal piece or knob of a tambour; the other depends on the application of a rubber cuff or armlet encircling the arm, attached to which is a hollow rubber ball of sufficient resilience to transmit the variations of pressure to the air within a surrounding glass sphere connected with a writing tambour. By this latter method, and a careful regulation of the pressure within the closed system so that it shall be maintained at a constant level between the diastolic and systolic pressure, a graphic record can be obtained, not only of the heart rate and rhythm, but also, within limits, of the variations of the intra-arterial pressure. This method becomes particularly valuable when clear, accurate records are desired of the pulse in conditions associated with pulsus alternans, or the vasomotor variations of pressure occurring with periodic breathing of the Cheyne-Stokes type.

Of the instruments using this closed air pressure system, that devised by Erlanger[1] record the variations with least instrumental variation. The smoked paper attachment of this apparatus, however, renders the whole so bulky, and adds so many difficulties, that few clinicians attempt to use it at the bedside.

The instruments depending for the recording impulse on the application to the artery of a metal lever or knob, require so much time to make the application and adjustment, and are so easily thrown out of adjustment, that a great share of the patience and attention of the operator is consumed in this adjustment. Often the conformation of the wrist of the patient is such that no record can be obtained in this way. These instruments are, for the most part, small, compact and

* Submitted for publication Jan. 29, 1916.

1. Erlanger, Joseph: A Criticism of the Uskoff Sphygmotonograph, THE ARCHIVES INT. MED., 1912, ix, 22.

fairly accurate. Thus, while one type of instrument has the advantage of small size and portability, the other has the advantage of ease and celerity in obtaining the arterial record.

There is, therefore, a place for a device which can be readily applied, which will remain adjusted with the least attention, which will record variations of intra-arterial pressure, and which can be employed with every other form of graphic machine. These advantages seem to be obtained in the capsule of the Erlanger instrument, and I have had made by Messrs. Schneider & Brothers, who made that instrument, a bulb modified as shown in the illustration. To obtain

Figure showing an adaptation of the Erlanger capsule for use with any polygraph to record the arterial pulse. The details are explained in the text.

records, the recording tambour of any polygraph is connected with the glass bulb at A by a tube in which there may be an equalizing valve. The tube B is connected with a leather covered armlet, while the tube C can be connected with any sphygmomanometer. The small inlet tube D, with the three-way stopcock, is used to control the supply of air to the closed system, which consists of rubber capsule in the glass chamber, armlet and sphygmomanometer. The stopcock is so arranged that by turning it as indicated to "on," "off," "out," the air can be pumped with an ordinary atomizer bulb into the tubes when the valve

is set at "on," and retained by turning the valve to "off," but allowed to escape by turning the key to "out." In the illustration, the protecting caps are shown screwed on the ends of the straight tube, but of course they must be removed to slip on the tubes connecting with the cuff and sphygmomanometer. The sphygmomanometer is necessary to learn the systolic and diastolic pressure, so that the pressure in the recording system may be maintained at a constant point when recording, and that the changes in arterial pressure may be registered. These changes occur most frequently in patients with Cheyne-Stokes type of breathing and with pulsus alternans. The instrument renders it easy to apply to an artery and retain in adjustment all polygraphs, makes it possible to take records with known pressure, and to obtain records showing maximum variations due to pulsus alternans and vasomotor changes.

158 West Fifty-Eighth Street.

A STUDY OF THE BLOOD IN HEMOPHILIA*

S. H. HURWITZ, M.D., AND W. P. LUCAS, M.D.
SAN FRANCISCO

INTRODUCTION

During the past few years the etiology of the hemorrhagic diseases has been the subject of considerable study. The progress made in this direction has been due largely to an increase in our knowledge of the physiology of blood coagulation and to the development of new methods of study. The literature of this period, indeed, has even given promise of an etiologic classification of hemorrhagic disease, but to the present time this goal has only been partly attained.

It has been possible, for instance, to demonstrate that certain of the hemorrhagic conditions are associated with a deficiency in one or another factor of coagulation: there are on record instances of hemorrhagic disease associated with abnormalities in the prothrombin and antithrombin content of the blood.[1] There is also experimental evidence of the existence of certain types of hepatic disease with some tendency to abnormal bleeding due to a deficiency in fibrinogen.[2] But on the other hand there are a number of clinical conditions characterized by a tendency to bleed, which at the present time cannot be rigidly classified on the basis of a defect in the fibrin factors.

Certain forms of purpura, for instance, are attributed by most workers to a deficiency in the number of blood platelets. Studies on the factors of coagulation in this disease have for the most part yielded negative results.[3] But repeated examinations of the blood of two patients with chronic purpura made over a period of six months have

* Submitted for publication Jan. 29, 1916.

* From the George Williams Hooper Foundation for Medical Research and the Pediatric Clinic, The University of California Medical School.

1. Whipple, G. H.: Hemorrhagic Disease. Antithrombin and Prothrombin Factors, THE ARCHIVES INT. MED., 1913, xii, 637. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, THE ARCHIVES INT. MED., 1914, xiii, 76. Drinker, C. K., and Hurwitz, S. H.: The Factors of Coagulation in Primary Pernicious Anemia, THE ARCHIVES INT. MED., 1915, xv, 733. Following the completion of the present paper, there appeared an extensive study by Minot, Denny and Davis (THE ARCHIVES INT. MED., 1916, xvii, 101) on the prothrombin and antithrombin factors in a large number of varied clinical conditions. Their observation that some types of purpura may show a high antithrombin content coincides with our experience.

2. Whipple, G. H.: Hemorrhagic Disease—Septicemia, Melena Neonatorum and Hepatic Cirrhosis, THE ARCHIVES INT. MED., 1912, ix, 365.

3. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, THE ARCHIVES INT. MED., 1914, xiii, 76. See also last paragraph of Note 1.

convinced us that certain chronic types of the disease may at times show blood abnormalities in addition to a platelet deficiency. Thus, in the patients studied by us, fluctuations were observed at different times in the amount of circulating antithrombin: periods of antithrombin excess occurred in waves during the course of the observations; but it could not be demonstrated that the increase in the amount of antithrombin was associated in any definite way with an aggravation in the severity of the clinical symptoms. These few observations are only suggestive, and from them alone no generalizations are permitted concerning the part played by the factors of coagulation in this type of hemorrhagic disease. It is not unlikely, however, that further studies may disclose other cases with a similar defect.

On the other hand, recent work has shown that the cause of hemophilia may be ascribed with considerable certainty to an abnormality in one of the factors of coagulation, and that the hemophilic condition is characterized by certain reactions of the blood which distinguish it from other hemorrhagic diseases. It is the purpose of this paper to present confirmatory evidence in support of this view.

VIEWS CONCERNING THE CAUSE OF HEMOPHILIA

The nature of the explanations offered for the causation of hemophilia varies with the theory of coagulation adopted. If we accept the theory of Howell, there are five factors concerned in the clotting of blood—prothrombin, antithrombin, thromboplastin, fibrinogen and calcium. All of these elements, except thromboplastin, are present in circulating blood. Within the blood vessels prothrombin is held in combination with antithrombin and intravascular clotting is thereby prevented. When, however, blood is shed, the antithrombin is neutralized by the thromboplastic substance of the tissue juices. The liberated prothrombin is now activated by the calcium and the thrombin which results converts soluble fibrinogen into the insoluble fibrin or the clot.

According to the opposing view of Morawitz, only four possible factors need be considered. The essential feature of this theory centers about the activation of thrombin, which, it is contended, results from prothrombin by the combined activity of calcium and a substance designated as thrombokinase. The existence of antithrombin in the circulating blood is admitted by Morawitz, but it is not regarded by him as an essential part of the process.

The main distinguishing feature of the hemophilic condition is the greatly prolonged coagulation time of the blood when it is removed from the vessels with the precautions necessary to prevent its contamination with tissue juices. All recent workers are agreed that this defect in coagulation is a constant pathological sign of the disease; but different explanations have been given by different workers to account

for the delayed coagulability. Hemophilia has been ascribed, indeed, to a deficiency in one or another of the various elements which take part in the clotting of normal blood. Without entering into a discussion of the experimental evidence given by each observer, it may be well to review briefly the essential fact of the various views presented.

Wright[4] has attributed the hemophilic condition to a deficiency of calcium. Weil[5] has given some experimental evidence for the view that the delayed coagulability of hemophilic blood may be caused by the presence of an excess of some coagulation inhibiting body. Sahli[6] and Morawitz and Lossen,[7] adopting the theory of coagulation proposed by Morawitz, have upheld the view that the essential defect in hemophilic blood may be ascribed to insufficient or defective formation of thrombokinase. This view is shared for the most part by Nolf,[8] who believes hemophilic blood to be deficient in the substance designated by him as thrombozym (thrombokinase, thromboplastic substance). Aldis,[9] however, has given experimental proof for the view that the hemophilic condition is due to a change in the properties of the circulating prothrombin, and Howell[3] has concluded that not the property but the actual amount of prothrombin is altered in hemophilic blood.

During the past year we have had an opportunity to make complete blood studies in five cases of hemophilia. These patients all exhibited the hemophilic condition in marked degree, although it was not possible to obtain a definite history of hemophilia in the ascendants. A study of the blood, however, showed in each instance the characteristic delay in the coagulation time and a striking deficiency in prothrombin. From the tabulated results it will be seen that the other factors of coagulation were present in normal amounts. With one or two exceptions, the absolute amount of antithrombin present in hemophilic blood was seldom greater or less than that of normal blood; and the amount of fibrinogen was found to fluctuate little from the normal.

Our conclusions support the view that the deficiency in prothrombin is a constant characteristic of the hemophilic condition. On the basis of this observation, it is essential that a definition of hemophilia not only include the clinical characteristics of excessive and immoderate hemorrhage and its transmissibility as a sex-limited inheritance, but also in addition the especial reaction of hemophilic blood which distinguishes it from the blood of other hemorrhagic diseases also characterized by a tendency to bleed.

4. Wright, A. E.: Brit. Med. Jour., 1893, ii, 223; ibid, 1894, ii, 57.
5. Weil, Emile P.: Presse méd., 1905, xiii, 673.
6. Sahli, H.: Ztschr. f. klin. Med., 1905, lvi, 264; Deutsch. Arch. f. klin. Med., 1910, xcix, 518.
7. Morawitz, P., and Lossen, J.: Deutsch. Arch. f. klin. Med., 1908, xciv, 110.
8. Nolf, P.: Ergebn. d. inn. Med., 1913, x, 275.
9. Addis, T.: Jour. Path. and Bact., 1911, xv, 427.

METHODS OF STUDY

1. *Determination of the Coagulation Time.*—The coagulation time of the blood was determined according to the method employed by Morawitz and Bierich[10] and by Howell. A specimen was obtained by venous puncture into a syringe coated with a thin layer of petrolatum-ether mixture. Of the sample obtained, 2 c.c. were expelled at once into a thoroughly cleaned test tube measuring about 13 to 14 mm. in internal diameter (the test tubes ordinarily used in bacteriologic laboratories serve well for this purpose). Tubes of the same diameter were used for all comparable determinations. The observations were made at a constant temperature (about 25 C). Complete invertibility of the clot was taken as the end point of the reaction. In each instance comparison was made with a specimen of blood taken from a normal individual with the same precautions. By this method the average clotting time of normal blood obtained in twenty-one tests was twenty minutes with a minimum of eight minutes and a maximum of thirty-three minutes.

It may be well to emphasize the importance of painstaking technic in obtaining blood for studies on the factors of coagulation, since a careless procedure may lead to erroneous results. In studies on the fibrin factors, interest centers on these elements as they exist in the circulating blood rather than on their behavior when blood is shed. The latter information is obtained better by a determination of the bleeding time,[11] the duration of which depends on a number of additional factors: the potency of the tissue juices, the mechanical and chemical action of the blood platelets, and the elasticity of the skin.

When blood is obtained by skin puncture, admixture with tissue juices invariably results. These exert an influence the extent of which cannot be measured, for it is obviously difficult to make certain that control specimens will be exposed to the same wound surface, and receive the same amount of contamination with thromboplastic substance. This point is well illustrated by an observation on the blood of one of Howell's[3] patients: the coagulation time of 2 c.c. of blood with which, owing to defective technic, some tissue juice was mixed, was 10 minutes, whereas the same amount of blood obtained at the same time directly from a vein had a coagulation time between four and five hours.

2. *Retraction of the Clot and Enumeration of Blood Platelets.*—It is well known that normally a blood clot quickly retracts from the

10. Morawitz, P., and Bierich, R.: Arch. f. exper. Path. u. Pharmakol., 1907, lvi, 115.

11. According to Duke (Jour. Am. Med. Assn., 1910, lv, 1185) the bleeding time is the tendency to bleed from a fresh cut. The duration of such a hemorrhage is determined by blotting up on filter paper all the blood which flows from a small incision at intervals of thirty seconds. Each drop will give, in a rough way, the volume of blood shed in the given time interval.

sides of the vessel in which it is contained and expresses serum. This phenomenon has been ascribed to the presence of the blood platelets. A deficiency in the number of these elements and nonretractility of the clot has been found to be a constant characteristic of some hemorrhagic conditions, notably purpura hemorrhagica. In both of these respects, most observers have reported hemophilic blood to be perfectly normal, but since observations on the character of clot retraction may be made during a determination of the coagulation time, and since the enumeration of blood platelets is not difficult, a record of both of these factors should be made, if possible, in all complete studies of the blood in hemorrhagic conditions.

The presence or absence of clot retraction is determined simply by incubating the clot at 37.5 C. for from twelve to twenty-four hours. In normal blood retraction begins after several hours, and is complete within eighteen to twenty-four hours; in pathologic blood, on the contrary, contraction may occur only after a long time or not at all.

For the enumeration of blood platelets a number of methods are available, but that of Wright and Kinnicutt[12] appears to have given the most reliable results in the hands of most workers. It has been our experience that successful counts can be obtained only with freshly prepared solutions and with perfectly clean apparatus.

3. *Quantitation of the Fibrin Factors—Method of Notation.*—The recorded observations include only determinations of prothrombin, antithrombin and fibrinogen. There is little of positive evidence in favor of the view that a deficiency of calcium exists in hemophilic blood (Morawitz and Lossen,[7] Nolf,[8] Addis,[9] and Hess[13]). A consideration of this factor, therefore, may be omitted in this study. According to the best experimental proof the existence of a thrombokinase in the sense of Morawitz is hypothetical. What is usually designated as thrombokinase in the blood and other tissues is the coagulation accelerating substance known under the terms of zymoplastic (Schmidt) or thromboplastic substance (Nolf and Howell). Whether or not this material is deficient in hemophilic plasma cannot be determined with any certainty because no direct method of studying this factor is available. Some information concerning this substance as it exists in the blood can be obtained by an enumeration of the platelets; for these elements, as will be recalled, are an important source of thromboplastic substance. But information obtained from this source is incomplete, inasmuch as all of the formed elements of the blood have been shown to possess thromboplastic properties to a greater or less extent.

12. Wright, J. H., and Kinnicutt, R.: A New Method of Counting the Blood Platelets, Jour. Am. Med. Assn., 1911, lvi, 1457.

13. Hess, A. F.: Bull. Johns Hopkins Hosp., 1915, xxvi, 372.

Prothrombin and Antithrombin.—The methods developed by Howell for the determination of prothrombin and antithrombin have been reviewed extensively in recent literature (Howell,[3] Drinker and Hurwitz[14]), and only brief reference to them will be made in this paper. The prothrombin deficiency which forms such a distinguishing feature of hemophilic blood, in contrast with other bloods, is demonstrated very clearly if to the specimen of clear plasma obtained by first oxalating and centrifugalizing the blood, calcium chlorid is added in optimum concentration. A specimen of normal blood so treated shows usually a coagulation time as measured by the invertibility of the clot of about ten minutes, whereas, the clotting time of hemophilic blood, treated in a similar manner, may be delayed from one to four or five hours.

Protocol 1 gives the results of one of the comparisons made between hemophilic and normal blood (Table 1), and shows the manner in which the reaction is carried out in practice.

PROTOCOL 1.—PROTHROMBIN TEST (CASE 3, NOV. 1, 1915)

Oxalated Plasma, Drops	$CaCl_2$ 0.5 Per Cent., Drops	Coagulation Time	
		Patient	Control
5	1	Clot in 24 hours.....	14 minutes
5	2	Partly clotted in 3 hours	12 minutes
5	3	Clot in 180 minutes.....	14 minutes
5	4	Partly clotted in 3 hours	14 minutes

The control specimen of blood in this particular experiment, it will be noted, clotted in twelve minutes. In order to indicate the extent of the delay in coagulation shown by the recalcified plasma, the term prothrombin quotient has been introduced. This is obtained by dividing the coagulation time of the specimen of pathologic plasma containing the optimum amount of calcium by the coagulation time of the normal blood tested at the same time and by the same method. In the present instance the prothrombin quotient is 180 divided by 12 or 15. This indicates in a rough way that in this particular experiment the coagulation time of hemophilic blood was fifteen times that of normal blood. In subsequent paragraphs it will be shown that this figure, however, has no especial clinical significance inasmuch as the gravity of the hemophilic condition has not been found proportional to the length of time required for the plasma to clot.

14. Drinker, C. K., and Hurwitz, S. H.: The Factors of Coagulation in Primary Pernicious Anemia, THE ARCHIVES INT. MED., 1915, xv, 733.

The method of determining the amount of antithrombin in hemophilic and in normal blood may likewise be rendered clearer by a specific example (Protocol 2).

PROTOCOL 2.—ANTITHROMBIN TEST[15]

Heated Plasma, Drops	Thrombin, Drops	Time Interval, Minutes	Fibrinogen, Drops	Coagulation Time, Minutes	
				Patient	Control
1	2	15	7	23	19
1	3	15	7	15	13
1	4	15	7	11	12
1	5	15	7	8	8
				57	52

ANTITHROMBIN QUOTIENT $= {}^{57}/_{52} = 1.1$

The use of the term antithrombin factor was suggested by Denny and Minot[17] to make the results obtained at different times and with different reagents comparable; but the term antithrombin quotient appears to us to be more appropriate. This quotient is obtained as shown above by dividing the average of a series of suitable determinations of the pathological blood by a similar figure obtained for a control specimen. It is obvious that if the antithrombin content of the patient's blood and that of a control are the same, the antithrombin quotient will be about unity. Slight fluctuations above or below one are within the experimental error and may be regarded as normal.

Fibrinogen.—Determinations of fibrinogen were made by the heat coagulation method described in a previous communication.[17] We are aware that this method of estimating fibrinogen is not beyond criticism, and that even normal individuals may show considerable variation in the amounts of fibrinogen determined in this way. In man, however, these fluctuations are not so great as in healthy animals. For clinical studies, therefore, this method is sufficiently accurate, especially if it be kept in mind that variations in the amounts of fibrinogen in disease in order to be of real significance should be of considerable magnitude.

4. *Quantitation of Serum Proteins.*—The availability of a simple and accurate method made possible a study of the different serum proteins in each of the five patients under observation. For this purpose the microrefractometric method recently described by Robertson[18] was

15. The solutions of thrombin and fibrinogen necessary for carrying out this test were prepared according to Howell's method (Howell, W. H., Am. Jour. Physiol., 1913, xxxii, 264).

16. Denny, G. P., and Minot, G. R.: Am. Jour. Physiol., 1915, xxxviii, 233.

17. Whipple, G. H., and Hurwitz, S. H.: Jour. Exper. Med., 1911, xiii, 136.

18. Robertson, T. B.: Jour. Biol. Chem., 1915, xxii, 233.

found very useful. The hemophilic blood is allowed to clot spontaneously or else coagulation is hastened by shaking with glass beads, and determinations are then made of the serum albumin, serum globulin, and total protein. The purpose of such a study would be to detect the presence of any abnormality in the protein partition products, should any arise during the clotting of pathological blood. So far as the authors are aware, this phase of the study of hemophilic blood has received no attention from previous workers.

5. *Fibrinolysis.*—The possible occurrence of fibrinolytic ferments in the blood of patients exhibiting hemorrhagic tendencies has been entertained by several observers. Such ferments have been demonstrated in some intoxications in animals and in man in certain types of leukemia and in hepatic insufficiency.[19] Morawitz and Lossen,[7] who studied this point in hemophilia, found no indication of fibrinolytic activity.

The test is easily carried out, and we believe should be done as a routine on every specimen of pathological blood examined. It is simply necessary to incubate the clot at body temperature, and to observe whether any dissolution occurs in the stated time interval. Since the clot from any specimen of blood will undergo a certain amount of dissolution if left in its serum a sufficient length of time, it is well to restrict the term pathological fibrinolysis to instances of complete clot dissolution occurring within twelve hours when the blood is kept at body temperature.

CLINICAL RECORDS AND BLOOD EXAMINATIONS

In a thorough analytical study of the hereditary aspects of hemophilia, Bulloch and Fildes[20] conclude that instances of probable hemophilia without demonstrated inheritance are comparatively few. It is their opinion that the introduction of the "de novo" concept is based on the inability of authors to demonstrate the line of inheritance in their cases. When it is remembered, however, that the other bleeders necessary to establish the inheritance of the condition may not be members of the same family, but of collateral branches, it is clear that the hereditary characteristics can be proved only with great difficulty.

It is fair to state that cases arising spontaneously or "de novo" without a history of bleeding in the family, have been recorded,[21] and

19. Goodpasture, E. W.: Bull. Johns Hopkins Hosp., 1914, xxv, 330.

20. Bulloch, W., and Fildes, P.: Eugenic Lab. Memoirs, Univ. of London, 1911, xii, 169.

21. Wright reports four cases without a hemophilic ancestry (Ref. 23, p. 926). Bulloch and Fildes give at length the history of a hemophilic family studied by Gettings, who was unable to find any instances of the disease among the collaterals, although these were widely inquired into over a number of generations (Ref. 20, pp. 191 and 343). In four of Schloessmann's (See Ref. 29) seven patients no history of heredity could be demonstrated, and this was true of one of Howell's patients.

that perhaps too much stress has been laid on the hereditary features. In the patients observed by us no definite history of hereditary transmission could be obtained, notwithstanding care in questioning, although it is not unlikely that the bleeding tendency existed somewhere in the direct ascendants or in the collateral branches of these families. But it is not justifiable to exclude such cases from the group of hemophilia, because there is no evidence of a similar condition in the family records. As has been shown by Howell, hemophilia is characterized by certain properties of the blood which distinguish it from other hemorrhagic diatheses. These, we believe, furnish a convenient method of making a diagnosis of hemophilia even in the absence of any proved history of the existence of the disease in the patient's antecedents.

CASE 1.—*Clinical History.*—(Hospital No. 6305). George R., aged 6 years. American born, of Swedish parents, was admitted to the University of California Hospital first in October, 1913, complaining of profuse and frequent nosebleed. Since then the patient has received treatment in this hospital at four different times (Hospital Nos. 6867, 8035, 9355, and 9572), each time for excessive hemorrhages associated with his hemophilic condition.

The patient's father was living and well, his mother was a healthy woman 44 years of age. According to her account she had had frequent attacks of epistaxis, and when 24 years of age had a profuse hemorrhage following the extraction of a tooth. She had noted that "black and blue" spots appeared occasionally on different parts of her body following slight trauma. Concerning the patient's ascendants, no definite information could be obtained; nothing was known of the paternal or maternal grandparents. The patient's aunt had four or five boys who were apparently free of any hemophilic taint. The patient had one sister 9 years of age who was in perfect health, and had at no time shown any signs of a hemophilic tendency, and, according to studies of her blood, there was no abnormality existing at the present time (M. R., Table 3). Two children had died when a few days old. The cause of their death was not known to the mother.

The patient was a full-term child; his development had been normal. The most important feature of his past history was an attack of pertussis at the age of 2½ years. The frequent hemorrhages from the nose, which form such a persistent symptom of his present condition, dated from this infection. In September, 1913, several weeks previous to his first admission for epistaxis, the patient cut his hand and bled for four days. Bleeding from the nose continued at intervals during his first four months' stay in the hospital. At times the hemorrhages were very severe and various forms of treatment were tried: two direct transfusions with little immediate or permanent effect on his condition; and the injection of human and rabbit's blood serum and of rabbit's whole blood both subcutaneously and locally to the bleeding points.

The patient was again admitted for profuse epistaxis twenty-two days following his discharge from the hospital. Two days after entrance he had a profuse hemorrhage from the stomach, and for the next few days passed tarry stools. So far as could be learned, this was the first sign of the gastro-intestinal hemorrhages which had occurred also during his last admission to the hospital. The treatment this time consisted simply of local applications of horse serum to the nasal mucosa. After a four months' stay (January, 1914, to May, 1914) the patient again returned in October, 1914, because of bleeding from a wound in the chin. In June, 1915, the occurrence of nosebleed and vomiting of blood necessitated the patient's readmission to the hospital. The gastro-intestinal hemorrhages, which had appeared first in January, 1914, had now recurred.

Examination and Clinical Course.—The positive physical findings were essentially the same during each admission: extreme yellow pigmentation of the skin, sclerae, and mucosae; a loud blowing systolic murmur and a palpable liver and spleen. During his first admission, a few small petechial hemorrhages were noted over the skin of the chest, abdomen and thigh; and during his last admission a note was made of small purpuric areas over both legs and of a similar area of large size about the left elbow.

This time the patient remained in the hospital about three months. On the first and second days following his admission, he received two subcutaneous injections of his father's blood, one of 6 c.c. and one of 20 c.c. Bleeding from the nose subsided, and the hemoglobin rose gradually from 15 to 40 per cent. (Dare). For a period of seventeen days (June 14 to June 30) the patient was fed on 0.1 gm. of kephalin daily.[22] This was supplemented by three subcutaneous injections of 0.05 gm. of kephalin. These injections caused a rise in temperature, and small areas of redness, infiltration, and local tenderness. From August 1 to 25 kephalin in doses of 0.1 gm. was again administered by mouth. This therapy was continued with the hope of modifying the coagulability of the blood, although the patient, at the time the treatment was undertaken, had no severe attacks of bleeding.

CLINICAL SUMMARY AND BLOOD EXAMINATIONS

Certain points in the history of this patient deserve special mention: first, the absence of a definite history of bleeding in the family; second, the appearance of the first signs of uncontrollable hemorrhage at the age of 2½ years following an attack of whooping cough, and their persistence with intermissions for a period of five years. In this connection it is of interest that the hemorrhages had been confined with few exceptions to the nasal mucosa, and that trauma had been a factor in their causation only twice; third, the absence of hemarthroses and articular effusions. This is in striking contrast to the clinical histories of Cases 2 and 3, which will be discussed later.

At different times during the patient's stay in the hospital and for a period following his discharge from the hospital, studies were made of the factors of coagulation and of the formed elements. For purposes of brevity, the separate protocols have been omitted and the results presented in Table 1. The first observations on the blood coagulability were made June 11, five days after a persistent nasal hemorrhage. The coagulation time was found markedly delayed, 335 minutes as compared with thirty-two minutes for a normal control. Five days previously the patient had received several small injections of whole blood, which apparently had not influenced his condition. The prothrombin, as will be noted from the table, showed the deficiency characteristic of this disease; the coagulation time of the recalcified plasma being about nine times as great as the control. Antithrombin was present in very slight excess. A second observation made September 2 following an interhemorrhagic period of two months, showed the same marked delay in coagulation, although to a less extent; but, as

22. The kephalin used in these studies was prepared according to the method outlined by Howell (Am. Jour. Physiol., 1912-1913, xxxi, 1).

will be noted, the deficiency in prothrombin was found to be actually more marked. This observation illustrates clearly that the extent of the delay in coagulation is demonstrated with greater accuracy if the blood is first oxalated and then recalcified. The antithrombin at this time was present in normal amount, and remained uninfluenced by the administration of kephalin during the months of July and August.

Six weeks after the patient's discharge from the hospital another examination of the blood was made (October 18). This observation is of extreme interest, because the blood obtained with the usual precautions and without admixture with tissue juices showed a more rapid coagulation time and a less striking prothrombin deficiency. Antithrombin was present in normal amount and the fibrinogen was slightly increased. It is difficult to explain this great increase in the coagulability of the blood. A similar improvement was noted also in Case 5, to which reference will be made later. It would appear that coincident with the waves of improvement observed clinically in these patients, there may also be some alteration in the coagulability of the blood. This improvement, however, was temporary, for two final observations, one on November 10 and another December 15, exhibited the same delay in coagulation and the same prothrombin deficiency as were recorded in June. The prothrombin observations in this patient showed that this factor is diminished in the interhemorrhagic interval as well as during the period of active hemorrhage. The observations indicate, however, that this deficiency in prothrombin may be less striking immediately following a period of profuse bleeding.

From the tabulated results, it is apparent that the retractility of the blood in this instance was normal. This is well in keeping with the persistence of a normal platelet count throughout the period of observation. These elements had been followed in this patient for over a year (Table 4) but, with two exceptions, they had been found normal or above normal. The table contains also observations on the number of white corpuscles and the relative percentage of each found in over twenty-five counts. It will be seen that the average number of leukocytes in this series was 2,150 and that of these 53 per cent. were polymorphonuclear elements. This is in harmony with the observations of Wright[23] and of Sahli,[6] both of whom found a subnormal number of leukocytes and a smaller percentage of polymorphonuclear cells in hemophilic blood.

No fibrinolytic activity was noted in the blood of this patient, and an analysis of the serum expressed from the clot after its slow formation showed a normal percentage of serum albumin and of serum globulin.

23. Wright, A. E.: Allbutt's System of Medicine, 1909, v, 935.

CASE 2.—*Clinical History.*—(Hospital Nos. 10055 and 10343). James McC., aged 6 years, was admitted to the University of California Hospital, October, 1915. Three days before admission the patient fell and struck his left eye. The wound thus received was still bleeding on admission.

Although the parents were carefully questioned, no history of any hemophilic tendency in the immediate ascendants could be obtained. The paternal grandfather was 78 years old and in good health; the paternal grandmother died of cancer at the age of 48 years. The maternal grandmother was 71 years

TABLE 1.—SUMMARY OF BLOOD

Case	Date	Hydrogen-Ion Conc. P H*	Coagulation Time, Minutes		Retractility of Clot	Fibrinolysis	Platelets (Thromboplastin)
			Patient	Control			
Case 1, G. R.	6/11	....	325	32	+	0	352,000
	9/12	7.8	195	15	+	0	.....
	10/18	....	23	16	++	0	296,000
	11/10	7.75	240	19	+	0	.....
	12/15	....	220	20	−	0	.....
Case 2, J. McC.	10/ 8	....	49	16	−	0	580,000 (Oct. 12)
	10/18	7.65	240	19	+−	0	.....
	10/21	....	...	..	±	...	192,000
	10/28	....	180	30	±	0	.....
	11/ 5	7.7	120	20	±	0	.....
	12/ 3	....	180	15	+	0	.....
	12/ 8	....	170	15	±	0	.....
	12/15	....	138	30	+	0	.....
	12/20	....	210	20	±	0	.....
Case 3, D. McC.	10/11	....	225	20	+	0	.....
	11/ 1	7.75	240	18	+	0	.....
	12/ 3	....	180	15	+	0	.....
	12/ 8	....	240	15	+	0	.....
	12/15	7.7	145	30	+	0	160,000
	12/20	....	240	20	+	0	.....

* The reaction of the blood was determined colorimetrically by the method proposed by Levy, Rowntree and Marriott (THE ARCHIVES INT. MED., 1915, xvi, 389).

of age; she had two brothers and five sisters, who were well. So far as could be learned, the sister's children were well, none having been troubled with bleeding. The patient's mother was of a family of two brothers and three sisters. The brothers were well. The one sister had two boys, both of whom were well.

The hemophilic tendency was first noted by the mother when the child was 3 months old. At that time she had noticed the appearance of "black and blue" swellings over the soles of the feet and the backs of the elbows following the slightest trauma. When 2 months old the child fell and struck his head. A large hematoma formed, which was opened. Bleeding from the wound continued for about two months and left the child extremely exsanguinated. At the age of 17 months the patient nicked his gum by the upper central

incisors and bled for a period of two weeks. At 2½ years of age, and again when 4 years of age, severe attacks of bleeding followed injuries above the right eye.

Joint effusions occurred repeatedly; the slightest trauma to an exposed joint having been followed by a painful swelling with areas of discoloration. The knees had suffered more than any other joints, and as a result of the repeated attacks these joints had become somewhat deformed, and showed considerable limitation of motion.

EXAMINATIONS IN CASES 1, 2 AND 3

Prothrombin			Antithrombin			Fibrinogen in Gm. per 100 c.c.	Treatment
Patient	Control	Quotient	Patient	Control	Quotient		
76	8	9.5	44	40	1.1	.....	Ten cubic centimeters whole blood given subcutaneously June 5, and 20 c.c. June 6. Kephalin 5 c.c. (0.05 gm.) subcutaneously on July 1, 4 and 7. Elevation of temperature to 40 C. and local reaction. Kephalin 0.1 gm. doses daily from August 1 to August 25.
180+	7	25 +	36	40	0.9	.....	
57	10	5.7	40	47	0.8	0.494	
...	..	...	...	...	...	.....	
160	17	9.4	61	82	0.8	.....	
60	14	4.3	28	20	1.4	.....	October 7 and 8 wound packed with sterile cotton soaked in 2.5 per cent. sterile kephalin and firm pressure applied. Twelve intramuscular injections of 5 c.c. kephalin (2.5 per cent. sterile solution) from October 7 to 15, and 9 intramuscular injections of 5 c.c. kephalin (1 per cent. in 6 per cent. glucose solution) from October 16 to 27. December 1 to 4 and 8 to 11 inclusive, exposure of tibiae and femora to Roentgen rays. Dose, 5 milliampere minutes.
195	10	19	37	47	0.8	0.420	
...	..	...	...	...	...	.....	
90	19	4.7	63	67	0.9	0.366	
60	10	6.0	...	...	...	.....	
85	15	5.6	91	38	2.3	.....	
72	25	2.8	103	103	1.0	.....	
115	17	6.7	50	99	0.5	.....	
200	14	14	130	149	0.9	.....	
150—	12	12 —	44	32	1.3	.....	December 1 to 4 and 8 to 11 inclusive, exposure of tibiae and femora to Roentgen rays. Dose, 5 milliampere minutes. Discontinued after this time because of low platelet count and prolongation of bleeding time.
180	12	15	57	52	1.1	0.250	
85	15	5.6	92	38	2.3	.....	
95	25	3.8	...	...	...	.....	
127	17	7.4	116	119	1.0	.....	
180	14	12	128	149	0.9	.....	

Examination and Clinical Course.—Apart from the condition in the knee joints the local injury was the chief feature in the physical examination. The left eye was swollen and completely closed; a large area of ecchymosis extended over both eyelids and over the upper part of the left cheek. Blood was oozing from the small incised wound, which, however, was found to be fairly deep.

Soon after admission the wound was cleaned and packed with gauze saturated in a 2½ per cent. solution of kephalin, which had been freshly prepared and sterilized. Some of the kephalin mixed with sterile vaselin was made into a paste and also packed into the wound. This treatment was repeated several times, and each time firm pressure was applied. By this means the external oozing was controlled within twelve hours, but some extravasation of blood still continued into the tissues as was apparent from the discoloration which spread over the whole of the left side of the face. Within

five days, however, the swelling and ecchymosis had commenced to disappear. On the sixth day, the pack was removed from the wound without the occurrence of any bleeding. As indicated in Table 1, the local application of kephalin was supplemented by intramuscular injections: twelve injections of 5 c.c. each of a 2½ per cent. solution of kephalin in water were given from October 7 to October 15; and nine such injections of a 1 per cent. solution in 6 per cent. dextrose, from October 16 to October 27. Notwithstanding the frequent repetition of these injections, no local induration or tenderness appeared, nor did the patient show any untoward constitutional symptoms.

Three weeks after admission a slight trauma of the right ankle caused a hemarthrosis which subsided within a week, but for which the patient was readmitted several weeks later. During this second admission, the effects of suberythema doses of Roentgen ray[24] on the factors of coagulation was tried. Exposure of the long bones was made daily from December 1 to 4 and from December 8 to 11 with an intermission of four days. The principles underlying this mode of treatment and its value in this disease will be discussed in subsequent paragraphs.

CASE 3.—*Clinical History.*—(Hospital No. 10345). Daniel McC., aged 9 years, brother of James McC., was admitted in November, 1915, complaining of swelling and pain in the left knee joint.

Both brothers gave strikingly similar histories; in the younger of the two, the symptoms of the disease had also appeared early in infancy. The mother stated that when the child was 3 months old, she had noticed for the first time bluish areas of discoloration over different parts of the body subjected to the slightest injury. When 11 months old, on first attempting to walk, the child injured his right knee. This injury, although not severe, caused an effusion into the joint. From that time to the present, considerable difficulty had been experienced with bleeding from minor accidents, three very severe hemorrhages having occurred between the ages of five and seven years: two of these followed cuts over the right and left eyes, and one followed an injury to the palm of the right hand, from which oozing continued for four months. Nearly every exposed joint in the body had been the site of an effusion. At the age of 4 years a fall on the left elbow resulted in complete disability for a period of a year. When 8 years of age, another severe hemorrhage followed the loosening of an incisor tooth.

Examination and Clinical Course.—Many ecchymotic areas of varying size were present over the arms and legs. These, together with the joint manifestations, constituted the chief findings on physical examination. All of the joints were found somewhat enlarged, but the knees were principally affected. The right knee was irregular in contour, and showed some impairment of motion. The left knee was swollen and tender. A few ecchymotic areas were present above and below the patella. Roentgenograms of the left knee showed marked distention of the synovial sac and some loss in definition of the articular surfaces. The latter presented the appearance of a chronic arthritis. Five days' rest in bed and moderate immobilization of the joint caused some absorption of the effusion and a subsidence of the pain; but the effusion returned following a repetition of the trauma, once fourteen days and again twenty-four days after admission.

In this patient also a study was made of the effect of short exposures to the Roentgen ray on the coagulability of the blood. Eight exposures of 5 milliampere minutes each were given, the interval between each four exposures being four days. At the end of this period the exposures were stopped because the reduction in the number of platelets and the prolongation of the bleeding time made it unwise to continue the treatment.

---

24. Exposure made at 12 inch target skin distance with Coolidge tube, 3 mm. aluminum filter, 6 inch parallel spark gap, 5 milliampere minutes, 1/20 erythema dose. We are much indebted to Dr. H. E. Ruggles of the University of California Hospital for making the exposures.

CLINICAL SUMMARY AND BLOOD EXAMINATIONS OF CASES 2 AND 3

The clinical histories of both brothers differed in several interesting points from that of Case 1. In them the first symptom of the hemophilic condition made its appearance in early infancy. The application of trauma was for the most part the initiating cause of the hemorrhages beneath the skin, from the open wounds, and into the synovial sacs. Repeated involvement of the joints, especially the knees, was one of the most striking symptoms in both patients, and in each instance these articular lesions resulted in chronic arthritis with deformity and disability.

The blood of each of these patients showed during every examination the characteristic prothrombin deficiency. It will be noted, however, that wide fluctuations in this factor were observed from time to time. These variations may best be considered in connection with the clinical condition and the form of treatment which was carried out. The first examination of the blood in Case 2 was made four days after the onset of bleeding (October 8). As the tabulated results indicate, the coagulability of the blood during this period was markedly increased. This is the only time that such a diminution in coagulation time during an attack of hemorrhage was observed in any of the patients. Increased coagulability of the blood, on the other hand, has been noted twice during the interhemorrhagic period (Cases 1 and 5). It would seem, therefore, that the contention of Sahli[6] that, during the hemorrhagic period hemophiliacs usually show a reduced or even a normal coagulation time, is not supported by our studies.

In other respects the blood of both brothers showed no abnormalities. Clot retraction was normal, and the percentages of the serum proteins and the plasma fibrinogen did not vary widely from values obtained for normal blood. With one exception, the antithrombin factor approximated one. In both patients a transitory antithrombin excess was observed about forty-eight hours following the commencement of exposure to the Roentgen rays. The effect of such exposures on the prothrombin-antithrombin balance will be discussed later.

CASE 4.—*Clinical History.*—Arthur S., aged 13 years, came to the dental clinic of the University of California in June, 1915, complaining of toothache. Because of his unusual history of repeated attacks of uncontrollable hemorrhage, Dr. C. R. Giles kindly referred him to us for observations on the coagulability of the blood.

So far as we could learn from the patient's mother, no bleeding tendency had existed in any members of her family. She had four sisters, all of whom had boys, but none of these had a similar trouble. She, herself, had lost one boy, aged 5 years, on account of some internal hemorrhage following an accident. But it was not possible to find out the exact circumstances attending this boy's death. It is of interest that this child was not circumcised because it was feared that the operation might result in an attack of uncontrollable bleeding.

The patient showed the first signs of the hemophilic tendency in early infancy. Circumcision performed ten days after birth was attended by profuse bleeding from the wound lasting for a number of days. From that time to the present great difficulty had been experienced with bleeding from minor accidents. When 1 year old the patient bit his tongue and bled profusely for six days. A similar accident to the gums two and a half years later again resulted in a profuse hemorrhage. This was unaffected by the application of styptics and the thermocautery. The first attack of epistaxis occurred at the age of 4 years, and since then the attacks have been frequent, especially in warm weather. Three very severe hemorrhages occurred between the ages of 6 and 10 years: the first followed an injury to the hand, which bled for over a week; the second and third attacks of bleeding were occasioned by extraction of teeth. Several months ago the patient again had four teeth extracted, and bled for seven to eight days.

CASE 5.—*Clinical History.*—Edward O., aged 11 months, came under observation in October, 1915. The patient was seen first by Dr. Florence M. Holsclaw, to whom we are much indebted for the opportunity of studying the blood and for the clinical notes.

TABLE 2.—SUMMARY OF BLOOD

Case	Date	Hydrogen-Ion Conc. + P H	Coagulation Time, Minutes		Retractility of Clot	Fibrinolysis
			Patient	Control		
Case 4, A. S.	6/21	...	150	23	+	0
	10/29	7.8	210	31	+	0
	11/20	7.7	150	30	+	0
Case 5, E. O.	10/26	...	305	30	+	0
	11/18	7.6	50	8	+	0
	12/15	...	...	..	....	...
	12/20	...	120	20	++	0

The baby was an illegitimate child, and because of this circumstance, information concerning the family history was not obtainable. The essential facts regarding the child's health since birth and the circumstances pertaining to the present illness were obtained from Dr. Holsclaw's records. From this account it was apparent that the child had been in perfect health before the onset of the present illness. A week before the baby came under observation, the nurse had noticed several "black and blue" spots, both large and small, scattered over the body. It was also noted that the child was fretful, nervous, and wakeful at night. Two days following the appearance of the ecchymoses on the body, similar ones were noticed over both cheeks, and a few days later (October 21) a large swelling involving the whole of the left cheek appeared. The skin over the swelling was discolored, tense, and shiny, and the veins over it were dilated. The interior of the mouth was dark purplish in color. There was also a bulging inside apparently due to an effusion of blood under the mucous membrane. Palpation of this swelling did not elicit pain, and there was no elevation of temperature.

The baby was taken to Lane Hospital, October 22, where the coagulation time of the blood was tested (Addis). This was only roughly estimated, since it seemed undesirable to puncture a vein or even to make a new skin puncture. Only blood oozing from a puncture in the big toe made some hours before

was available. Capillary tubes were filled with this blood, and these were used for estimating the coagulation time. In two tubes times of seventeen and twenty minutes were observed as compared with a normal of seven minutes.

During the next few days the swelling and ecchymosis of the left cheek had begun to disappear, and bleeding from the needle prick made in the big toe three days previously (October 23) was at length stopped by pressure. October 26 the child was brought to the University of California Hospital where venipuncture was done for a study of the factors of coagulation. Two similar examinations were made subsequently, one November 18 and another December 20. At the time the patient was seen last, the swelling of the cheek had almost entirely disappeared, the "black and blue" spots had faded, and the child's general condition was excellent.

EXAMINATION OF THE BLOOD

The main points derived from a study of the blood of both of these patients have been presented in Table 2. In a general way the findings in these cases correspond to those recorded in Table 1, but it may be

EXAMINATIONS IN CASES 4 AND 5

Platelets (Thrombo-plastin)	Prothrombin			Antithrombin			Fibrinogen in Gm. per 100 c.c.
	Patient	Control	Quotient	Patient	Control	Quotient	
200,000	73	9	8	14	17	0.8	0.378
.....	90	16	6	43	37	1.1	0.425
352,000	120	12	10	31	28	1.1	0.390
64,000	170	19	9	50	53	1.0	.....
.....	80	17	5	120	90	1.3	.....
248,000	...	..	..	...	...	...	.....
296,000	200	14	14	170	149	1.1	.....

well to call attention to several features which deserve special consideration.

During the period of observation, the fourth patient had no attacks of bleeding, but the blood showed, nevertheless, the characteristic delay in coagulability and a marked prothrombin deficiency. All of the other elements, as will be noted, were normal. The first observation in Case 5, on the other hand, was made only a few days after the onset of hemorrhage, and while the patient was still bleeding. It will be observed that the coagulation time was markedly prolonged, and the prothrombin much reduced in amount. During a subsequent examination, however, made about three weeks later, when active bleeding had entirely ceased, great improvement was noted also in the condition of the blood; but as is characteristic of this disease, the increase in blood coagulability was only temporary, for a third observation a month later showed a return to the initial condition.

The observations on the blood platelets in this patient are of extreme interest. It will be recalled that a small skin puncture, made several days after the onset of the symptoms, bled for about three days notwithstanding the application of firm pressure. This extreme prolongation of the bleeding time is well in keeping with the low platelet count (64,000) obtained at this period. Enumeration of these elements about two months later, when all signs of acute hemorrhage had disappeared, showed them to be normal. It is justifiable to conclude, therefore, that the extreme prolongation of the bleeding time was due in this instance to a deficiency in blood platelets. None of the other patients observed showed such a marked platelet deficiency; and it would appear that its occurrence is rare in hemophilia, although reduction in the number of

TABLE 3.—RESULTS OF EXAMINATION OF BLOOD OF SISTER OF CASE 1 AND OF MOTHER AND SISTERS OF CASES 2 AND 3

Case	Member of Family	Coagulation Time, Minutes		Prothrombin			Antithrombin		
		Patient	Control	Patient	Control	Quotient	Patient	Control	Quotient
1	Sister (M. R.).....	23	—	9	10	0.9	17	19	0.9
2	Sister (Agnes McC.)....	23	20	12	9	1.3	58	49	1.1
	Sister (Adeline McC.)..	20	23	12	10	1.2	52	50	1.0
	Mother.....	16	19	14	10	1.4	20	25	0.8

these elements has been noted by some observers.[25] Of interest also is the fact that the prothrombin remained low even when the platelets had returned to a normal level. The relation of blood platelets to the origin of prothrombin will be considered in another connection.

DISCUSSION OF RESULTS

Apart from the special abnormality in the fibrin factors to which reference has been made, a study of the blood of our hemophilic patients showed that hemophilic and normal bloods differ in no other respect. According to our observations, a change in the activity of the fibrin factors is not due to any alteration in the reaction of the blood, for we have found the hydrogen-ion concentration of hemophilic blood serum to vary little from that of normal blood. There also is no abnormality in clot retractility, which constitutes the third phase of coagulation. In this respect hemophilia differs from some other hemorrhagic

25. Austin, J. H., and Pepper, O. H. P.: Experimental Observations on the Coagulation of Oxalated Plasma, with a Study of Some Cases of Purpura, THE ARCHIVES INT. MED., 1913, xi, 305.

conditions, notably purpura, in which this phase of coagulation has been found abnormal. The clot in hemophilic blood is slow to form, but it possesses normal retractile power; in fact, in several instances, the retraction of the clot and the expression of serum was greater in hemophilic than in normal blood.

In still another particular, hemophilic blood has been found normal. An analysis of the serum after coagulation has shown that the percentage of serum albumin and serum globulin approximates very closely that of normal human blood. It is true, as the tabulated results show (Table 6), that the average percentage content of total proteins is somewhat diminished in hemophilic blood, and that the globulin fraction shows a slight relative excess, but these differences are too small to possess real significance. On the whole, the results indicate that no abnormality in the protein partition products arises during the clotting of hemophilic blood, and that the change observed in the fibrin factors cannot be brought into association with any alteration in the serum proteins.

As already pointed out, our observations support the view of Howell that the essential cause of the delayed coagulability of hemophilic blood is attributable to a deficiency in the circulating prothrombin. The other factors of coagulation have been followed. Gravimetric determinations of the fibrinogen content of hemophilic blood have convinced us that no abnormality exists in this element. This finding is in harmony with those of Sahli,[6] who weighed the fibrin produced by the coagulation of hemophilic blood, and found it to be within the limits of normal variation. That no qualitative defect in this factor exists has been shown by Addis.[9] By the addition of varying amounts of thrombin to fibrinogen prepared from hemophilic blood and from normal blood, he was able to demonstrate that the former clots as rapidly as the latter. It is possible to conclude from the available experimental evidence, therefore, that no qualitative or quantitative defect exists in the fibrinogen of hemophilic plasma.

According to our results, the amount of antithrombin in hemophilic blood also exhibits little variation from the normal. For the most part, the antithrombin quotient was only slightly above or below one. Such fluctuations as were observed may be considered to lie within the experimental error, for the quotient seldom showed variations from one greater than several tenths. It is of interest that a definite excess of antithrombin was demonstrable in the blood of two patients about forty-eight hours after the commencement of Roentgen-ray exposures (Cases 2 and 3). This rise, however, was only transitory, and was no longer present five days later. These results do not support the view held by some observers that the delayed coagulability of hemophilic blood is due to an absolute excess of antithrombin;[5] nor are they in harmony

with the experiments presented by other workers to prove that hemophilic blood contains less of this coagulation-inhibiting substance.[7] On the whole, our work supports the conclusion that there is no marked difference between the absolute amount of antithrombin contained in hemophilic and in normal blood plasma, although it is clear that this

TABLE 4.—Record of Leukocytic and Platelet Counts from October, 1913, to October, 1914, in Case 1

Date	Platelets*	W. B. C.	Polymorphonuclears, Per Cent.	Large Round, Per Cent.	Lymphocytes, Per Cent.	Eosinophils, Per Cent.	Remarks
10/ 9/13	280,000 (W. & K.) 211,000 (P.)	3,900	62.6 67	3.6 2	31.8 31	1	
10/14/13	400,000 (W. & K.)	6,400	67	6	26	1	Bleeding time 1 min. 5 sec.
10/16/13	350,000						
10/20/13	860,000?	8,100	54	10	36		
10/23/13	456,000	7,000	60.4	4	35	0.6	Bleeding time 3 min. 20 sec.
11/ 4/13	324,000	5,200	59.6	4.3	36.1		
11/12/13	.....	4,600	45	18	36		
11/13/13	.....	6,700	59	5	34	1	
11/15/13	.....	6,000	65	5	29		
11/17/13	416,000	6,400	63	7	30		
11/19/13	.....	5,100	46	21	30	1	
11/21/13	.....	3,600	49	17	33		
11/24/13	.....	3,400	41.5	8.6	48.4	1	
12/ 1/13	.....	7,200	47	4	47	2	
12/ 8/13	.....	8,200	46	11	43		
12/18/13	.....	7,000	50	10	40		
12/28/13	.....	4,200	40	5	55		
1/ 6/14	.....	3,400	38	4	58		
2/ 2/14	.....	8,400	60	7.5	30		
2/13/14	372,000	6,200	54	15	26		
2/20/14	256,000	6,125	43	31	23	1	Bleeding time 5 min.
3/ 8/14	.....	5,380	40	10	46	3	
3/20/14	184,000	7,400	55	15	28		
4/ 2/14	160,000						
10/ 9/14	.....	11,400	53	5	34	1	Bleeding time 4½ min.
10/18/14	.....	10,300	65	7.5	26	2	
Average	.....	2,150	53				

* The method of enumerating the platelets used is given wherever the exact technic employed is stated in the records. W. and K. means Wright and Kinnicutt's method, P. means Pratt's method. For the most part one or the other of these two methods was used.

substance must be present in relative excess in hemophilia in proportion to the extent of diminution of the available prothrombin.

The deficiency in the amount of prothrombin was demonstrated by the simple procedure already described of oxalating the plasma and then adding to it the optimum amount of calcium. Our studies make it possible to state with confidence that this procedure furnishes a reliable means of differentiating hemophilic blood from the blood of other hemorrhagic conditions. Although this element has been followed throughout various periods of the disease, at no time has it been found normal. It has been our experience that wide fluctuations

TABLE 5.—Record of Leukocytes and Platelets, Cases 2 and 3

Case 2 (J. McC.)

Date	Platelets	W. B. C.	Polymorphonuclears, Per Cent.	Large Round, Per Cent.	Lymphocytes, Per Cent.	Eosinophils, Per Cent.	Remarks
11/24/15	.....	8,200	70	9	21	..	Roentgen-ray exposures commenced 12/1 and ended 12/11.
12/ 3/15	216,000	15,200	53	10	33	2	
12/13/15	.....	11,800	64	12	24	1	
12/15/15	160,000	.....	..	..	..	..	Bleeding time prolonged.

Case 3 (D. McC.)

10/10/15	.....	12,900	94	15	4	..	Roentgen-ray exposures commenced 12/1 and ended 12/11.
10/15/15	580,000	11,150	56	10	32	3	
10/22/15	192,000	.....	70	8	21	1	
11/24/15	.....	10,400	52	10	34	4	
12/ 3/15	.....	13,000	53	6	40	2	
12/13/15	.....	14,600	72	7	20		
12/15/15	204,000						

in the amount of prothrombin may exist during the hemorrhagic as well as during the interhemorrhagic periods of the disease, and that the degree of this defect bears no definite relationship to the severity of the clinical symptoms. This point is well illustrated by the observations in Cases 1 and 2. In both of these patients a less marked reduction of the circulating prothrombin was noted immediately after an attack of bleeding, but a similar increase in this factor has been observed also during the interhemorrhagic period. On the basis of the experimental evidence at hand, it is therefore impossible to make any definite statements concerning this point.

Although it can be stated with a fair degree of certainty that the proximate cause of the delayed coagulability is due to a diminution in prothrombin, the ultimate cause of the condition is still a matter of speculation. Some knowledge concerning this more fundamental problem can be gained from the studies on the origin of prothrombin. So far as is known at present, the circulating prothrombin of the blood plasma is furnished by the blood platelets. This fact is based on good experimental evidence. But it is difficult to bring these observations into harmony with the well-known fact that the number of blood platelets in hemophilia is normal. In explanation of this paradox, it has been assumed that the defect in question is attributable to a functional rather than to a numerical change in these elements. This assumption has received some experimental support from the recent work of Fonio.[26] As this worker has shown, platelets derived from hemophilic blood are less potent in hastening the clotting of such blood than are platelets obtained from normal blood. Fonio refers this defect of hemophilic platelets to a diminished thrombozym (thromboplastin) content; but it would appear that the evidence presented does not

TABLE 6.—ALBUMIN, GLOBULIN, AND NONPROTEIN IN HEMOPHILIC BLOOD SERUM

Patient	Date	Total Protein Per Cent.	Total Albumin Per Cent.	Total Globulin Per Cent.	Albumin, Per Cent. of Total Protein	Globulin, Per Cent. of Total Protein	Nonprotein Constituents, Pre Cent.
G. R., Case 1.....	11/10	7.3	5.4	1.9	74	26	1.0
	12/15	6.6	5.1	1.5	76	24	1.2
J. McC., Case 2.....	10/28	6.9	5.2	1.7	75	25	1.1
	11/ 5	6.6	4.8	1.8	73	27	1.2
	12/ 3	6.4	5.3	1.1	82	18	1.0
D. McC., Case 3.....	10/11	7.1	5.0	2.1	70	30	1.0
	11/ 1	7.0	4.2	2.8	60	40	1.0
	12/ 3	7.0	4.5	2.5	64	36	1.0
	12/15	7.6	5.0	2.6	65	35	1.1
A. S., Case 4.....	10/29	7.2	5.3	1.9	73	27	1.0
	11/20	7.3	5.3	2.0	73	27	1.1
E. O., Case 5.....	11/18	6.7	5.9	0.8	88	12	1.0
	12/20	6.6	5.5	1.1	82	18	1.0
Averages.....	.....	6.9	5.1	1.8	73	26	1.0
Normal human serum*	.....	7.94	6.20	1.74	78	22	1.09

* The figures for the percentage concentration of serum albumin and serum globulin in normal human blood serum are the averages obtained by Tranter and Rowe (Jour. Am. Med. Assn., 1915, lxv, 1433) on about thirty normal serums.

26. Fonio, A.: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, xxviii, 313.

establish with any certainty that this functional defect of hemophilic platelets may not be due to a diminution in their prothrombin content. For, as is well known, the solution of platelets when blood is shed facilitates clotting in two ways: first, by setting free prothrombin; and second, by liberating a thromboplastic substance which hastens coagulation by neutralizing the antithrombin present normally in the circulating blood.

Furthermore, in the solution of this paradox the possibility must be kept in mind that other factors besides the blood platelets may be concerned with the origin of prothrombin. For instance, it has been shown that although a considerable diminution in prothrombin may result from the destruction of myeloid tissue following benzol injections, no direct parallelism exists between this drop in prothrombin, the number of platelets, and the extent of bone marrow injury.[27] These experimental observations support the view that the maintenance of the prothrombin equilibrium of the blood depends only in part on the blood platelets. At the present time it is not possible to state what tissue or tissues besides the marrow may be concerned in the elaboration of prothrombin. Thus far experiments have given no support for or against the contention of Nolf[6] that liver cell activity is essential for prothrombin production.

THE EFFECT OF KEPHALIN AND OF THE ROENTGEN RAYS ON THE BLOOD IN HEMOPHILIA

Studies concerning the proximate cause of the delayed coagulability of hemophilic blood naturally suggest the idea that it might be possible to devise some means of supplying to the patient the element in which his blood is deficient. A rational therapy of this kind might be approached theoretically in one of three ways: first, by a readjustment of the prothrombin-antithrombin balance to a more normal state by the introduction of thrombin or its antecedent substance, prothrombin, directly into the circulation; second, by stimulating the tissues concerned with prothrombin elaboration to form more of this deficient factor; and lastly, by a neutralization of the relative excess of antithrombin by the injection of tissue extracts. Some attempts have already been made to modify the coagulability of the blood of healthy animals by means of some of these methods. Experiments of this nature have developed the very interesting fact that in health the blood or the body can protect itself within wide limits from the effects tof such injections. For instance, Davis,[28] working in Howell's laboratory, has shown that solutions of pure thrombin may be introduced

27. Hurwitz, S. H., and Drinker, C. K.: Jour. Exper. Med., 1915, xxi, 401.
28. Davis, D.: Am. Jour. Physiol., 1911-1912, xxix, 160.

into the circulation in quantities that in the test tube would cause rapid and firm clotting and yet no harm be done. It is apparent, therefore, that a healthy animal can protect itself from the effects of substances which would endanger its life by the formation of intravascular clots. The nature of this defensive reaction consists, it is thought, in the formation within the organism of a compensatory amount of antithrombin to bind either the injected thrombin or to neutralize the tissue extracts introduced into the circulation. But we do not know with any certainty whether or not the same response can occur in pathological states in which the balance is already disturbed in the direction of an increased or decreased coagulability. Indeed, only few attempts have been made to modify by these means the blood in diseased conditions associated with a defect in the fibrin factors. Because of the meagerness of data on this point, it seemed worth while to study this phase of the subject on some of the patients under observation.

On the basis of some experiments which will be recorded in another paper, we studied the influence produced by the injections of kephalin and the effect of Roentgen irradiation on the blood in hemophilia. With regard to kephalin, preliminary experiments on healthy rabbits had shown that this substance could be introduced into the circulation either intramuscularly or intravenously without any untoward symptoms, and that the immediate effect of intravenous injections was an increase in the coagulability of the blood. This increased coagulability was only transitory, however, and was followed soon by a return of the blood to a normal state, or in a few instances by a decreased coagulability. The exposure of animals to suberythema doses of Roentgen rays, on the contrary, produced no perceptible increase in coagulability, although an examination of the blood for the separate factors disclosed a transitory rise in the circulating prothrombin. The temporary character of this reaction and its failure to decrease the coagulation time can be explained in the light of the defensive power of the organism considered in a previous paragraph. And although these experiments showed that the effect of the administration of kephalin and of exposure to the Roentgen rays produced only temporary changes in the condition of the blood, the possibility still remained that these agents might act differently in conditions like hemophilia. Accordingly we decided to try the effects of one or both of these measures on three of the patients.

In the first patient observations were made on the effect of kephalin therapy alone; whereas, both the second and third patients were treated with kephalin and with the Roentgen rays. Concerning the dosage of kephalin, the preliminary experimental work had assured us that a considerable amount of this substance could be administered intra-

venously without any untoward symptoms. In some animals as much as 10 c.c. of a 1 per cent. solution were introduced into the ear vein without ill effects. Although some workers[29] have investigated the coagulation accelerating effect of intravenous injections of saline extracts in hemophilia, we did not feel that such injections were entirely devoid of danger, and we have, therefore, no data to present on this point. It will be noted from the clinical records that only the oral, subcutaneous and intramuscular modes of giving kephalin were used. The first method was early abandoned because it proved ineffective, and subcutaneous injections were discarded because they were painful. Intramuscular injections, on the other hand, were not followed by any local or constitutional reaction, and these were used exclusively in the second and third patients.

In the choice of the proper dosage for the Roentgen-ray exposure, we were guided largely by following the formed elements in the blood. As is well known from the work of recent observers,[30] small and large doses of Roentgen rays have antagonistic effects on the blood-forming organs; the former exert a stimulating action and the latter a destructive action. For our purposes, it was determined that one-twentieth of an erythema dose applied to the long bones at the stated intervals produced the desired stimulation of the blood-forming organs as evidenced by the rise in leukocytes. In one of the patients (Case 3), a drop in the platelet count and a prolongation of the bleeding time after the eighth exposure showed that the rays were beginning to act destructively, and this served as an indication for stopping the treatment.

The results of both of these forms of therapy have been presented for the most part in the clinical histories and in the tabulated results. Only a few points deserve further consideration. In order to avoid criticism, it is desirable in the first place that judgment regarding the results of any therapeutic measure used to control hemorrhage be guided by some criteria. Apart from all other considerations, it seems fair to assume that a therapeutic measure is successful only if it causes a cessation of the bleeding, and so affects the disease process itself that the coagulability of the blood is increased. On the basis of such criteria, it is not possible to make any great claims for Roentgen-ray therapy and for kephalin injections in the constitutional treatment of hemophilia. Concerning Roentgen-ray treatment, it is safe to conclude that small stimulating doses do not alter in any way the condition of the blood in hemophilia. It is not possible to say at

29. Schloessmann, H.: Beitr. z. klin. Chir., 1912, lxxix, 492.

30. Heinecke, A.: Deutsch. Ztschr. f. Chir., 1905, lxxviii, 196. Duke, W. W.: Variation in the Platelet Count, Its Cause and Clinical Significance, Jour. Am. Med. Assn., 1915, lxv, 1600.

present whether these negative results in hemophilia, in contrast with those obtained in healthy animals, are due to a lack of response on the part of the blood-forming organs or whether more prolonged and more intensive treatment is necessary to effect any changes.

Furthermore, the especial therapeutic use of kephalin has not been found to lie in its influence on the disease process, for we have not been able to show that this substance administered intramuscularly in the dosage employed by us effects any noticeable change either in the coagulability of the blood or in the prothrombin-antithrombin balance. Only once did the opportunity arise to determine the value of intramuscular injections in the control of external bleeding (Case 2). But in this instance, it will be recalled, the injections were combined with the application of kephalin to the wound surface. Because similar injections in other patients had failed of effect, we are inclined to attribute the early arrest of bleeding in this case to the local action of the kephalin. Of its great hemostatic properties we have become convinced from observations on its coagulation accelerating action on whole blood in the test tube as well as when applied locally to bleeding surfaces. And although similar properties have been claimed for other tissue extracts, we believe that its thermostability gives kephalin a decided advantage over saline or aqueous extracts in that it can be sterilized without weakening its action.[31] It is to be hoped, therefore, that kephalin will prove of real value in the treatment of uncontrollable hemorrhages from external wounds so frequently met with in hemorrhagic conditions.

SUMMARY

1. No alteration has been observed in the reaction of hemophilic blood. In all of the patients studied, the hydrogen-ion concentration of the serum showed the normal variation.

2. No abnormality exists in the third phase of coagulation: clot formation is slow, but the clot, when once formed, shows normal retractile power.

3. The percentages of serum albumin, serum globulin, and total protein of hemophilic serum do not show wide variations from the normal.

4. The essential defect of hemophilic blood, which accounts for its delayed coagulability, is a diminution of the circulating prothrombin. The other two fibrin factors, antithrombin and fibrinogen, are present in normal amounts. Wide fluctuations may be observed in the pro-

31. Howell has called attention to this property of saline or aqueous extracts. He has shown that heating such solutions precipitates the protein, together with the active phosphatid. But if the phosphatid is first extracted with ether, the residue may be dissolved in water and the solution is unaffected by boiling (Am. Jour. Physiol., 1912-1913, xxxi, 1).

thrombin content of hemophilic plasma both during the hemorrhagic and interhemorrhagic periods. No definite relationship can be shown to exist between the extent of the prothrombin deficiency and the gravity of the clinical symptoms.

5. The method suggested by Howell of first oxalating the blood and then recalcifying with an optimum amount of calcium gives a simple and reliable means of diagnosing hemophilia and of differentiating it from other hemorrhagic conditions. It is to be recommended that this test be carried out preliminary to any operation on a patient exhibiting the hemophilic tendency.

6. Oral, subcutaneous and intramuscular injections of kephalin have no effect on the disease process,

7. Kephalin applied locally to the bleeding wounds of hemophilics brings hemorrhage to an early arrest. Because of its great hemostatic properties and its thermostability kephalin deserves an important place in the treatment of bleeding from external wounds.

8. Suberythema doses of Roentgen rays do not influence the disease process: no alteration was noted either in the coagulability of the blood or in the prothrombin-antithrombin balance.

Second and Parnassus Avenues—University Hospital.

THE SIGNIFICANCE OF THE URIC ACID, UREA AND CREATININ OF THE BLOOD IN NEPHRITIS*

VICTOR C. MYERS, PH.D., AND MORRIS S. FINE, PH.D
WITH THE COOPERATION OF
WALTER G. LOUGH, M.D.
NEW YORK

In an earlier communication[1] attention was called to the practical value of the estimation of the creatinin of the blood in nephritis. It was pointed out that an appreciable retention of creatinin indicated a grave impairment in the functional condition of the kidney, for the reason that creatinin is normally the most readily eliminated of the three nitrogenous waste products—uric acid, urea and creatinin. In contrast to creatinin, however, uric acid is apparently eliminated by the kidney with difficulty. It is, therefore, not surprising that conditions of moderately decreased kidney permeability should be encountered, in which only the concentration of the uric acid of the blood should be raised. This appears to be the case in gout and early interstitial nephritis. In the present paper it is our intention to lay emphasis on those cases in which the permeability of the kidney is not sufficiently impaired to cause a marked retention of creatinin.

The deductions which Sir A. B. Garrod[2] drew from his work in this connection in 1848 are very interesting, and their general harmony with current views surprising when one considers the methods then available. His conclusions (referring to Bright's disease and the albuminuria following scarlatina) were:

> 1. Uric acid is always present in the blood in albuminuria. The quantity, however, greatly varies: when the functions of the kidneys are much impaired, it exists in quantities almost as great as in gout; in other cases, its amount is small, but it usually exceeds that found in ordinary blood. 2. Urea always exists in large quantities in this blood (a fact which has long since been proved) and no relation is found between the amounts of urea and uric acid. 3. The kidneys are always deficient in their power of throwing off urea; but with regard to uric acid, their excreting function may be impaired or not.

*Submitted for publication Feb. 11, 1916.

*From the Laboratory of Pathological Chemistry and the Medical Service of the New York Postgraduate Medical School and Hospital.

1. Myers, V. C., and Lough, W. G.: The Creatinin of the Blood in Nephritis: Its Diagnostic Value. THE ARCHIVES INT. MED., 1915, xvi, 536.

2. Garrod, A. B.: Observations on Certain Pathological Conditions of the Blood and Urine in Gout, Rheumatism and Bright's Disease. Medical-Chirurgical Trans., 1848, xxxi, 83.

Garrod's paper apparently aroused considerable discussion, for he appended the following:

> Postscript July 26, 1848. At the discussion which ensued after the reading of the above paper to the society, some remarks were made which implied that I was understood as considering gout to be entirely dependent upon the power of the kidney for the excretion of uric acid; such, however, is not my opinion, and at present I do not wish to advance any hypothesis as to the cause and nature of gout, considering that many further researches should be made on the subject before a theory of the disease could be advanced with safety.

The investigation of von Jaksch[3] in 1896 gave confirmation to the observations of Garrod on the retention of uric acid in nephritis.

Von Noorden[4] gives a most interesting discussion on the uric acid content of the blood in gout and nephritis. He writes:

> The primary cause of the uric acid retention in gout lies in the kidney, according to Garrod. Recently Levison and Luff, and, with certain reservations, also Strauss, have advocated the same view. Levison and Luff showed, in a very large series of postmortems in Denmark and in England, that in cases of interstitial nephritis, uric acid deposits in the articular cartilages are very frequently found, although the condition was not suspected during life. Stripped of all unnecessary detail, this means that the retention of uric acid in the blood and the remaining phenomena of gout are the results of primary disease of the kidney. In certain cases both nephritis and gout are amenable to clinical diagnosis. In other cases, nephritis, and in still others the uric acid diathesis, remain for a long time, perhaps even up to the end of life, clinically free from symptoms and unrecognized. . . .
>
> The figures of Levison and Luff show, in the first place, that nephritis and gout occur in the same individual more frequently than was formerly assumed. They recall to us the facts that harmful influences, especially alcoholism and saturnism, and also heredity, play an acknowledged part in the etiology of both diseases. . . .
>
> . . . It appears that continuous overloading of the blood with uric acid (nephritis) does not necessarily lead to gout, and, moreover, that this overloading is not followed by uric acid deposition without the accession of another still unknown, specific gouty factor.

No noteworthy advance in this connection, however, was made until the advent of Folin's new method.[5] The work of Folin and Denis,[6] and others employing their methods, has been principally to confirm the views advanced by Garrod nearly seventy years ago, although with the quantitative data now available more definite conclusions have been possible. Folin and Denis and the authors[7] have reported cases of uremia with marked retention of uric acid, urea and creatinin. Twelve cases have come under our observation with more than 10 mg. of uric

3. Von Jaksch, R.: Beitrag zur Kenntnis der Uricacidämie der Nephritiker. Zentralbl. f. inn. Med., 1896, xvii, 545.
4. Von Noorden, C.: Metabolism and Practical Medicine, 1907, iii, 669.
5. Folin, O., and Denis, W.: Jour. Biol. Chem., 1913, xiii, 469.
6. Folin and Denis: Jour. Biol. Chem., 1913, xiv, 29; 1914, xvii, 487.
7. Myers, V. C., and Fine, M. S.: Jour. Biol. Chem., 1915, xx, 391.

acid per 100 c.c. of blood, in one case 27 mg.; still no gouty symptoms were evident. It is of interest regarding the uric acid that early in the disease the values observed may be somewhat higher (7 to 8 mg.) than at a later stage (5 to 6 mg.), although during the last days of life the amount may very markedly increase, this being coincident with an accumulation of creatin in the blood.

Folin and Denis[8] noted that in the severest cases of uremia there was only a slight increase in the blood ammonia, and that it was likewise only these cases in which a marked retention of creatinin occurred. From this they conclude:

> The figures obtained indicate that the human kidney removes the creatinin from the blood with remarkable ease and certainty. The completeness of the creatinin excretion is, in fact, exceeded only by the still more complete removal of the ammonium salts.

It is possible that other factors, such as acidosis and the formation of urea, may have some relation to the increase in the ammonia of the blood, but the increased blood creatinin is obviously due to one cause, namely, the markedly impaired permeability of the kidneys. Since creatinin is the most readily eliminated of the nitrogenous waste products (creatinin, urea and uric acid) and uric acid the most difficultly eliminated, as indicated above, urea must obviously stand in an intermediate position (see Table 3). That this should be the case seems quite plausible when we consider the ease of excretion of these constituents as determined from a comparative nitrogen partition of normal urine and blood. Uric acid nitrogen forms 2 per cent. of the nonprotein nitrogen of both urine and blood, urea nitrogen about 85 per cent. in urine, but 50 per cent. in blood, and creatinin nitrogen 5 per cent. in urine, but only 2 per cent. in blood. It is quite possible that the physical properties and concentration of these constituents in the blood may play an important rôle in the ease with which the kidney eliminates them.

It has been possible to select from a large series of miscellaneous blood analyses a considerable number of cases with decidedly high figures for uric acid, but with only slightly increased urea concentrations and with creatinin figures scarcely above the normal limits. As was pointed out in a previous communication,[1] a creatinin of over 5 mg. per 100 c.c. of blood appears to indicate an early fatal termination. It was the object of the present study to secure cases of the same type, but at an earlier stage in the disease, when changes in diet and mode of living might be of some aid in withholding for a time the uremia, from which most of these patients ultimately die.

---

8. Folin and Denis: Jour. Biol. Chem., 1914, xvii, 487.

METHODS EMPLOYED

Before proceeding to a discussion of our data on this subject, it is fitting that a brief description should be given of the methods we have employed for the estimation of uric acid, urea and creatinin in blood. Folin has recently raised certain objections to his own technic[9] for the estimation of uric acid in blood. We have embodied the suggestions of Benedict[10] in our application of the method, and are confident of the reliability of the results. The Duboscq colorimeter has generally been employed for the estimation of the uric acid and creatinin, although in the case of urea, the Hellige instrument has frequently been used. For the estimation about 20 c.c. of oxalated blood are needed. To avoid complications from the influence of food and possible changes in blood volume, the blood has generally been taken in the morning before breakfast.

Uric Acid.—Ten cubic centimeters of the well-mixed blood are pipetted into a casserole of about 375 c.c. capacity and approximately 5 volumes of hundredth-normal acetic acid added. The mixture is heated over a water bath and finally brought to a boil over a free flame, stirring continuously. About 4 c.c. of fairly thick alumina cream[11] are added with continuous stirring for a few minutes. The sides of the dish are washed down from time to time with hot water and the mixture then filtered through a hardened filter paper. The coagulum is returned to the casserole with about 150 c.c. of hot water, heated and filtered through the same paper. The combined filtrates are evaporated to 1 or 2 c.c. (the material should be protein-free) and transferred to a 15 c.c. conical centrifuge tube, washing the casserole with 2 or 3 small portions of hot water but keeping the volume at or below 10 c.c.

About 15 drops of ammoniacal-silver-magnesium mixture[12] are now added, the tube shaken and placed in a cool place (refrigerator) for about 15 minutes to allow for the precipitation of the purins. The tube is centrifuged, the supernatant liquid decanted off and allowed to rest in inverted position for about five minutes. The tip of the tube is then wiped with filter paper and the ammonia allowed to volatilize (may be facilitated with suction).

For the development of the color prepare a 100 c.c. graduated cylinder for the standard and a 50 c.c. cylinder for the unknown. Five c.c. of the uric acid standard[13] (5 c.c. = 1 mg. of uric acid) are pipetted into the 100 c.c. cylinder.

9. Folin, O., and Bell, R. D.: Proc. Am. Soc. Biol. Chem., 1915.

10. Benedict, S. R.: Jour. Biol. Chem., 1915, xx, 629.

11. Prepared by precipitating an 8 per cent. solution of aluminum acetate in acetic acid with sodium bicarbonate, carefully washing with a large volume of distilled water by decantation several times, then filtering.

12. The ammoniacal-silver-magnesium solution is prepared by mixing 70 c.c. of 3 per cent. silver nitrate solution, 30 c.c. of magnesia mixture and 100 c.c. concentrated ammonia. Any turbidity which may develop is filtered off. Magnesia mixture is prepared by dissolving 35 gm. magnesium sulphate and 70 gm. ammonium chlorid in 280 c.c. distilled water and then adding 140 c.c. concentrated ammonium hydroxid.

13. The standard uric acid solution is prepared as follows: Dissolve 9 gm. pure crystallin hydrogen disodium phosphate and 1 gm. dihydrogen sodium phosphate in 200 to 300 c.c. hot water. Filter and make up to about 500 c.c. with hot water. Pour this warm, clear solution on 200 mg. uric acid suspended in a few cubic centimeters of water in a liter volumetric flask. Agitate until *completely* dissolved. Add at once exactly 1.4 c.c. glacial acetic acid. Make up to 1 liter, mix and add 5 c.c. chloroform. Five cubic centimeters of this solution are equivalent to 1 mg. uric acid. The solution should be freshly prepared every two months.

To the standard are added 2 drops of 5 per cent. potassium cyanid, 2 c.c. of Folin-Macallum regent,[14] 20 c.c. of saturated (22 per cent.) sodium carbonate, and, in about one minute, water to the 100 c.c. mark. To the precipitate in the centrifuge tube are added 1 or 2 drops of the potassium cyanid, 2 c.c. of the Folin-Macallum reagent and 15 or 20 c.c. of the saturated sodium carbonate, depending on whether the color is subsequently diluted to 50 or 100 c.c. After forty to sixty seconds dilute with water until the intensity of the color is similar to the standard and then match in the Duboscq colorimeter. The prism of the standard may conveniently be set at the 10 mm. mark.

Urea.—The method is based on the suggestions of Marshall[15] and Van Slyke.[16] Into a test tube (of such size that it will just slip into a 100 c.c. cylinder) are introduced 1 c.c. of 10 per cent. urease solution, or about 0.1 gm. of the dry enzyme,[17] and then 1 to 2 c.c. of water. Two cubic centimeters of blood are now added with an Oswald-Folin pipet and the tube incubated in a beaker of water at 50 C. for about one-half hour. At the end of this time 3 to 4 drops of caprylic alcohol or 1 c.c. of amyl alcohol are added to prevent foaming in subsequent aeration. Into a 100 c.c. graduated cylinder, without lip, are added 15 c.c. of distilled water and 2 drops of 10 per cent. hydrochloric acid. This is now closed with a two-hole stopper having a glass tube passing nearly to the bottom of the graduate. The tube is sealed at the lower end, but contains a number of small holes to aid in the complete absorption of the ammonia (Folin). To the test tube containing the digested blood is carefully added an equal volume of saturated sodium carbonate, or better still, potassium carbonate, the solution being allowed to run underneath the blood. The tube is now immediately inserted in a 100 c.c. (ungraduated) cylinder and a two-hole stopper is used containing one glass tube which passes nearly to the bottom of the tube. This is now connected on one side with a wash bottle containing dilute sulphuric acid and on the other with the graduated cylinder containing the dilute acid for the absorption of the ammonia. The ammonia of the blood is now transferred to the cylinder containing the weak acid, by vigorous aeration for twenty to thirty minutes. At the end of this time the outfit is disconnected and nesslerization (Folin) carried out in the graduated cylinder, dilution being made according to the amount of ammonia present.

Into a volumetric flask of 100 c.c. capacity, if the Duboscq colorimeter is to be used, are pipetted 5 c.c. of ammonium sulphate or ammonium chlorid solution containing 1 mg. of nitrogen.[18] About 50 to 60 c.c. of distilled water are next added. Ten cubic centimeters of modified Nessler's solution[19] are now diluted about five times with distilled water, and of this 20 to 25 c.c. added to the standard solution, which is then made up to the mark with water. At the

14. The reagent, as modified by Prof. S. R. Benedict (private communication), is prepared by boiling 100 gm. sodium tungstate, 20 c.c. concentrated hydrochloric acid and 30 c.c. 85 per cent. phosphoric acid in 750 c.c. distilled water for two hours, preferably under a reflex condenser, and then making up to 1,000 c.c. with water.

15. Marshal, E. K.: Jour. Biol. Chem., 1913, xv, 487.

16. Van Slyke, D. D., and Cullen, G. E.: Jour. Biol. Chem., 1914, xix, 211.

17. Prepared by Dr. I. F. Harris, Bronxville, N. Y.

18. The solution may be prepared by dissolving 0.944 gm. ammonium sulphate or 0.764 gm. ammonium chlorid of the highest purity in distilled water and making up to 1,000 c.c.

19. For the modified Nessler's solution, place 100 gm. mercuric iodid and 50 gm. potassium iodid, both finely powdered, in a liter volumetric flask and add about 400 c.c. water. Now dissolve 200 gm. potassium hydroxid in about 500 c.c. water, cool thoroughly and add with constant shaking to the mixture in the flask; then make up with water to the liter mark. This usually becomes perfectly clear. Keep at body temperature over night, or until the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of dark brownish red precipitate remains. The solution is now ready to be siphoned off and used.

same time 7 to 8 c.c. of the freshly diluted Nessler's solution are added to the unknown solution and the volume made up to 25 c.c. in the graduate, unless a high content of urea nitrogen is indicated, in which case more Nessler's solution (up to 25 c.c.) and a dilution to 33⅓, 50, 100 c.c. or even more may be needed to make the color of the unknown of approximately the same intensity as the standard. The colorimetric readings should be made without delay, the standard prism being set at the 10 or 15 mm. mark.

Creatinin.—The technic for the creatinin estimation is briefly[1] as follows: 5 c.c. of the well-mixed blood are treated with 20 c.c. of water (4 volumes) in a 50 c.c. centrifuge tube. After the corpuscles have been laked, about 1 gm. of dry picric acid is added, and the mixture stirred at intervals with a glass rod until it is a light yellow. When the protein precipitation is complete, the tube is centrifuged and the supernatant fluid filtered through a small filter paper. To 10 c.c. of the filtrate is added 0.5 c.c. of 10 per cent. sodium hydroxid, and a similar amount of alkali added to 10 c.c. of standard creatinin in saturated picric acid.[20] It is best to make up simultaneously three standards containing 0.3, 0.5 and 1.0 mg. creatinin to 100 c.c. of picric acid. The tube corresponding most nearly with the unknown is used as standard, the prism of the Duboscq colorimeter being set at either the 10 or 15 mm. mark. Allow ten minutes for the color to develop before comparing.

DISCUSSION

Although considerable attention has recently been given to the urea and nonprotein nitrogen of the blood in nephritis, scant consideration has been accorded the uric acid and creatinin. It is true that the greater part of the waste nitrogen is eliminated in the form of urea, but it does not necessarily follow from this that data on uric acid and creatinin are unimportant and uninteresting. In fact, Folin's[21] classic work of ten years ago on the urine, by way of comparison, would suggest that the reverse might be true. Outside of Folin's laboratory and our own, practically no work has been reported in which estimations of the uric acid, urea and creatinin have been simultaneously carried out on pathological bloods. We do not believe that it is possible to make satisfactory deductions regarding nitrogen retention from the urea or nonprotein nitrogen determinations alone, as many have recently done.

In the most recent paper of Folin and Denis[22] dealing with blood uric acid, these authors point out the importance, from a diagnostic standpoint, of having the uric acid estimation accompanied by that of nonprotein or urea nitrogen. Their data give figures for nonprotein nitrogen and uric acid. It seems unfortunate to us that they did not include figures for urea and creatinin. The determination of the urea concentration of the blood is, we believe, of more value than that of the nonprotein nitrogen, for the reason that it represents the reten-

20. Creatinin may now readily be prepared perfectly pure by the admirable method of Benedict, Jour. Biol. Chem., 1914, xxiii, 183.

21. Folin, O.: Am. Jour. Physiol., 1905, xiii, 45.

22. Folin, O., and Denis, W.: The Diagnostic Value of Uric Acid Determinations in Blood, The Archives Int. Med., 1915, xvi, 33.

TABLE 1.—Blood Analyses in "Early Interstitial Nephritis"

Date 1915-16	Case	Age	Sex*	Blood Analysis			Phthalein 2-Hour Output	Blood Pressure		Urine		Remarks
				Uric Acid, Mg. to 100 c.c.	Urea N, Mg. to 100 c.c.	Creatinin, Mg. to 100 c.c.		Systolic	Diastolic	Albumin	Casts	
8/11	1. J. Ja.	65	♂	9.5	25	2.5	13	185	90	+	+	Apparently early chronic interstitial nephritis.
9/25				8.4	27	2.7		150	85			
1/14				5.0	37	3.9		130	...			
9/21	2. D. D.	52	♂	8.7	20	3.6	20	100	87	+	+	Cirrhosis of liver and chronic interstitial nephritis; severe alcoholism.
9/14	3. L. G.	28	♂	7.2	22	2.1	..	130	95	—	+	Pulmonary tuberculosis.
9/22	4. H. J.	60	♂	7.2	18	2.5	..	...	...	+	—	Prostatic hypertrophy.
6/ 7	5. D. S.	56	♂	7.1	16	2.0	26	185	110	—	+	Apparently early chronic interstitial nephritis.
7/21				6.6	24	3.3						
9/28				6.3	18	2.1	43					
8/13	6. J. Ju.	58	♂	7.0	23	2.6	..	115	80	+	— pus	Chronic constipation; alcoholism.
11/16	7. A. R.	57	♂	6.8	20	1.8	40	140	80	—	+	Carcinoma of stomach; metastases to liver; chronic interstitial nephritis; pneumonia four months previous to admission; painful urination; loss of weight.
11/30				6.3	22	2.5						
6/15	8. M. S.	41	♀	6.7	22	1.5	53	120	75	++	—	Frontal headaches; scarlet fever and diphtheria as a child.
9/14	9. G. P.	27	♂	6.7	14	2.4	62	130	90	+	+	Typhoid fever; headaches.
9/17	10. H. L.	23	♂	6.5	16	2.7	58	130	90	++	+	Pulmonary tuberculosis; tuberculosis of kidney; frequent urination; pain in bladder; headache; dizziness and fainting spells.
8/ 3	11. C. Ma.	54	♂	6.3	31	2.9	45	150	90	—	—	Hypothyroidism; chronic interstitial nephritis; increasing weight; pain in lumbar region.
8/31				4.2	20	1.9						
9/21				6.3	23	2.4						

10/ 8	12. J. Co.	69	♂	6.3	40	1.5	20	130	80	+++	+	Endocarditis; chronic interstitial nephritis (?); cystitis; edema of feet and chest.
9/14	13. M. R.	69	♂	6.3	15	2.5	..	128	84	++	—	Myocarditis; dyspnea; edema of legs; pneumonia two years ago; moderate alcoholism.
9/10	14. Ja. C.	69	♂	6.0	36	2.1	50	...	...	+++	—	Prostatic hypertrophy.
10/18	15. L. J.	59	♂	5.8	45	3.6	Trace	128	85	+	— pus	Tabes dorsalis; pyelitis; impairment of vision.
10/26	16. J. T.	55	♂	5.6	30	1.6	33	118	85	+	+	Diabetes; cataract of right eye; mild chronic interstitial nephritis.
8/10	17. E. H.	41	♂	5.6	13	2.1	45	150	65	—	—	Pericarditis; edema of feet and ankles; moderate alcoholism.
9/21	18. S. S.	47	♂	5.5	25	3.0	..	135	95	++	+	Lobar pneumonia; scarlet fever as a child; periodic drinker.
6/11	19. T. S.	49	♂	5.5	24	3.3	62	150	85	+	+	Carcinoma of larynx; chronic interstitial nephritis; chancre 29 years ago; Wassermann —.
10/12	20. F. D.	45	♂	5.5	12	2.5	37	185	120	—	+	Chronic interstitial nephritis; pain in back, radiating to right thigh; mild grade of optic neuritis.
8/ 3	21. F. S.	20	♀	5.5	11	2.7	40	130	95	++	++	Acute parenchymatous nephritis, following administraton of salvarsan four months previously; generalized edema; frontal headaches; Wassermann ++.
4/15	22. C. Mo.	54	♂	5.4	15	2.3	36	212	164	+	+	Chronic diffuse nephritis; myocardial insufficiency; Wassermann ++++; edema of abdomen and chest.
4/27				3.6	16	2.9						
9/ 7	23. S. P.	24	♀	5.2	15	2.5	60	130	92	+	— pus	Syphilis; fainting spells; headaches.
10/26	24. J. A.	32	♂	5.0	21	2.0	28	140	60	+	+	Chronic endocarditis; arthritis; palpitation of heart; dyspnea.
11/ 5	25. F. T.	47	♀	5.0	14	1.4	52	160	90	—	—	Hyperthyroidism; general weakness; loss of weight; bronzing of skin; scarlet fever as a child; typhoid fever.
11/19	26. K. W.	49	♀	5.0	22	2.5	28	225	140	++	+	Chronic endocarditis; chronic interstitial nephritis; decreased urinary output; dyspnea; scarlet fever as child.
12/ 8				4.2	19	1.6	37					

* ♂ signifies male; ♀ signifies female.

tion of a definite compound, and, further, is more easily and more accurately determined. Where possible, however, both determinations are desirable. Folin and Denis divide their cases into the four mathematically possible groups, namely, cases with the uric acid and nonprotein nitrogen of the blood, both normal; cases with normal uric acid and high nonprotein nitrogen; cases with high uric acid and normal nonprotein nitrogen (gout); and cases with high figures for both uric acid and nonprotein nitrogen (nephritis). The classification they have employed is obviously valuable, although we believe the "staircase effect" of the retention of uric acid, urea and creatinin, brought out in our Table 3 is even more valuable, particularly as regards the so-called uric acid bloods. In agreement with Folin and Denis, we have likewise observed cases with moderately high figures for nonprotein and urea nitrogen but with normal figures for uric acid. These cases belong to quite a different group (some of them are cases of parenchymatous nephritis), and will not be discussed at present.

It will be evident from the present paper that there are many cases which have only a slight or moderate retention of urea, but in which there is a very marked retention of uric acid (Table 3, Groups I and II). We might refer to other cases in which there was present, in addition, a marked retention of urea, but in which the creatinin retention was comparatively slight (Table 3, Group III). In the cases of the latter group the prognosis from the urea alone would have been far different from that made in the light of the blood creatinin. Still other cases might be mentioned in which a greater retention of creatinin was present and proved the more valuable diagnostic sign.[1] The determination of uric acid, urea and creatinin obviously gives a survey of the character of nitrogenous retention that would not be possible from the urea alone. Other tests may, of course, prove of value. Thus, in many cases of severe nephritis, the acidosis may be a factor of greater moment than the nitrogen retention, as we have recently observed with the aid of Van Slyke's apparatus for determining the carbon dioxid combining power of the blood.

It may be well at this point to recall that the normal range of these nonprotein nitrogenous blood constituents is, for uric acid, 2 to 3 mg., for urea nitrogen, 12 to 15 mg., and for creatinin, 1 to 2.5 mg. per 100 c.c. of blood. An inspection of Tables 1 and 2 shows uric acid figures between 5 and 10 mg. In most instances the figures for urea nitrogen are likewise increased, the majority of observations being between 20 and 30 mg. per 100 c.c. of blood. The urea retention is, however, nothing like that encountered in more advanced cases of interstitial nephritis, as shown in Table 3. The figures for creatinin, on the other hand, are nearly all very close to, or only slightly above, the normal limits.

The data of Table 1 give the results of a series of twenty-six cases[23] in which the concentration of the blood uric acid is decidedly increased without a corresponding increase in the urea or creatinin. Some of these cases show symptoms which, in general, are characteristic of "early interstitial nephritis." In other cases, although the nephritis was not the predominant clinical condition, it would appear that the systemic disturbances resulting from, or associated with, a variety of conditions, such as tuberculosis, typhoid fever, pneumonia,

TABLE 2.—Evidence of "Interstitial Nephritis" in Four Cases of Diabetes (Three Fatal) as Shown by the Examination of the Blood[24]

Date 1915-16	Case	Age	Sex	Sugar of Blood, Per Cent.	Sugar of Urine, Per Cent.	Uric Acid, Mg. per 100 c.c. of Blood	Urea N, Mg. per 100 c.c. of Blood	Creatinin, Mg. per 100 c.c. of Blood	CO_2* Combining Power of Plasma, c.c. per 100 c.c.
10/29	L. F.[1]	52	♀	0.80	2.2	10.5	55	2.1	37
10/30				1.10	0.5	...	..	...	31
4/10	M. W.[2]	53	♂	0.37	1.7	6.0	18	2.0	..
4/17				0.98	1.6	...	..	...	..
2/ 4	M. S.[3]	38	♀	0.53	1.3	5.0	44	2.3	49
8/26	B. S.[4]	46	♀	0.42	3.6	7.6	28	4.7	12

* Corrected values.

1. Duration of disease, several years; no coma until day of death, October 30; small amount of albumin in urine of October 26; 2.7 per cent. of sugar; only faint trace of acetone in urine.

2. Gangrene of toes at times during the last five years; glucosuria very severe early in the disease but later improved; systolic blood pressure 150 to 165 mm.; phthalein output 44 per cent.; moderate amount of albumin in urine of April 10; at death (April 18) urine showed only small amount of acetone; uremic symptoms thirty hours before death and about six hours previous to last blood analysis.

3. Shortness of breath; vomiting; increased frequency of urination; cloudy urine; history of scarlet fever; blood pressure 125 to 85; urine showed from faint trace to small amount of albumin; sugar, 1.0 to 1.5 per cent.; trace of acetone; no casts found; phthalein output 13 per cent.

4. Patient entered hospital in coma and died several hours later; urine contained very large amounts of albumin, acetone and diacetic acid, and many granular casts.

carcinoma, cardiac disorders, chronic alcoholism, etc., exerted the same influence on the kidney. It is not improbable that similar factors are at work in gout and the apparently uncomplicated cases of interstitial nephritis.

The data recorded in Table 2 are likewise of interest in this connection, since they clearly demonstrate a similar state of affairs in four

23. For the opportunity of studying the majority of these cases we are under obligations to the director of the Medical Department, Dr. Edward Quintard. We are also indebted to Drs. Samuel Lloyd, J. Bentley Squier, Arthur F. Chace and Robert H. Halsey for a number of interesting cases which they have brought to our attention.

TABLE 3.—Uric Acid, Urea N and Creatinin of Blood in Gout and Various Stages of Nephritis

Date 1915-16	Case	Age	Sex*	Diagnosis	Present Condition	Uric Acid, Mg. to 100 c.c. of Blood	Urea N, Mg. to 100 c.c. of Blood	Creatinin, Mg. to 100 c.c. of Blood	Phthalein Output 2 Hrs.	Systolic Blood Pressure	Urine	
											Albumin	Casts
9/ 3	M. K.	49	♀	I Typical case of gout	Unchanged	9.5	13	1.1	48	230	+	—
10/ 5	T. B.	57	♂			8.4	12	2.2	35	164	—	+
10/ 6	L. J.	43	♂			7.2	17	2.4	..	200	—	—
10/ 6	C. P.	..	♂			6.8	14	1.7	..	...	..	..
8/11	J. Ja.	65	♂	II Apparently early cases of chronic interstitial nephritis	Unchanged	9.5	25	2.5	..	185	+	+
9/25						8.0	37	2.7	18	150		
1/14						5.0	37	3.9	..	130		
6/ 7	D. S.	56	♂			7.1	16	2.0	..	...	—	+
7/21						6.0	24	3.3	26	185		
9/28						6.3	18	2.1	43	...		
9/21	D. D.	52	♂			8.7	20	3.6	20	100	+	+
8/13	J. Ju.	58	♂			7.0	23	2.6	..	175	+	—
8/ 3	C. M.	54	♂			6.3	31	2.1	23	150	—	—
1/ 6	L. P.	57	♂			8.0	80	4.8	0	240	++	++
3/ 1						4.9	17	2.9	10	170		

4/23	J. P.	34	♂	III Moderately severe cases of chronic interstitial and chronic diffuse nephritis	Improved	8.3	72	3.2	25	238	+++	++
5/21						5.3	21	1.9	43	145		
1/15	W. C.	49	♂			9.5	44	3.5	28	210	++	++
1/28						2.5	19	1.9	52	120		
8/ 6	L. B.	32	♀			7.7	67	3.1	..	165	++	+
10/ 1						6.7	17	1.6				
8/ 7	L. H.	30	♀			8.3	38	2.9	10	200	+++	++
8/24						6.5	24	3.0				
4/11	E. C.	50	♀	IV Typical terminal cases of chronic interstitial nephritis with uremia	Died	22.4	236	16.7	0	210	++	Pus
3/25	T. D.	34	♂			15.0	240	20.5	2-3	225	++	+
1/25	S. H.	37	♂			14.3	263	22.2	0	220	++	+
5/28	M. O.	8	♀			13.0	90	11.1	0	265	+++	+
4/15	J. W.	34	♂			6.7	144	11.0	Trace	225	+	+

* ♂ signifies male; ♀ signifies female.

The cases in Group I require no further comment here, while for Group II reference may be made to the same cases in Table 1.

The following remarks may be made concerning the cases in Group III:

L. P.—Chronic interstitial nephritis with uremia; marked edema of feet and ankles previous to admission; thickening of arterial walls; left hospital improved.

J. P.—Chronic diffuse nephritis; Wassermann ++++; generalized edema; left hospital improved.

W. C.—Chronic diffuse nephritis; chronic alcoholism; severe headache; mild generalized edema; probable edema of brain; severe acidosis on admission as shown by carbon dioxid combining power of blood (Van Slyke); improving.

L. B.—Acute parenchymatous nephritis; pulmonary tuberculosis; pericarditis; edema of feet, ankles and face; left hospital improved.

L. H.—Chronic interstitial nephritis; mitral regurgitation; dyspnea; occipital headaches; left hospital improved.

The cases in Group IV need no comment. The blood analyses reported were those made shortly preceding death.

advanced cases of diabetes.[24] In the first case it will be noted that the uric acid reached 10.5 mg. and the urea 55 mg. per 100 c.c. of blood, although the creatinin figure was quite normal.

The results collected in Table 3 summarize observations illustrative of those on which our views on this subject have been based. Typical cases of gout show, as a rule, blood uric acid values from two to five times the normal (Group I). The amounts of urea and creatinin are normal, or in the case of urea only slightly above normal. Many early cases of nephritis, probably of the interstitial type, give blood pictures which differ little from those of gout[25] (Group II). The uric acid findings are quite as high and the urea content varies from only slightly above to more than double the normal amount. The creatinin is only slightly increased. As the condition of the cases of this type becomes more severe, the retention of urea increases, until we have high values for urea as well as for uric acid (Group III). If improvement takes place, the concentration of urea gradually falls until the picture is that of the preceding group. If, on the other hand, the case goes on to a fatal termination, the retention of uric acid and urea is followed by that of creatinin, the concentration of which may reach twenty times the normal (Group IV).

SUMMARY

A series of thirty cases are recorded with high values for the uric acid of the blood, but without a corresponding retention of urea and creatinin. These cases were apparently suffering from "early interstitial nephritis," probably secondary in many instances to other systemic disturbances. Since the blood urea was not markedly increased or the phthalein output markedly decreased (in certain cases), it is believed that the uric acid was of considerable value as an early diagnostic test. The possibility is further suggested that a retention of uric acid may be earlier evidence of renal impairment of an interstitial type than the classical tests of albuminuria and cylinduria.

The blood pictures in early interstitial nephritis and gout are strikingly similar, particularly as regards the increase in uric acid. In view of the other clinical signs in common, it would seem that this similarity must be more than accidental.

---

24. Some of these cases have already received attention in another connection (blood sugar); Cf. Myers, V. C., and Bailey, C. V.: Jour. Biol. Chem., 1916, xxiv, 147.

25. The relation of uric acid to gout will be made the topic of a future communication from this laboratory by one of us (M. S. F.) and Dr. C. V. Bailey.

From the data in Table 3 it is evident that as the permeability of the kidney is lowered in the type of cases studied, it becomes apparent, first, by a retention of uric acid, later by that of urea, and lastly, by creatinin, producing a "staircase effect."

THE EFFECTS OF SUPRARENAL FEEDING*

R. G. HOSKINS, PH.D.
Associate Professor of Physiology, Northwestern University Medical School
AND
AUGUSTA D. HOSKINS, A.M.
CHICAGO

Within recent years several careful studies have been made of the effects of feeding different ductless gland substances. So far as we are aware, however, the suprarenal has received little attention. The matter seemed worthy of investigation for two reasons: Suprarenal gland substance is still on trial in practical therapeutics. Although many observers have been unable to detect in it significant therapeutic virtues, others claim more fortunate results. Williams,[1] for example, values it highly in the treatment of so-called neurasthenia, which he maintains is frequently a form of adrenal deficiency. In a case of impotency in the male, Belfield[2] has reported striking improvement following the use of desiccated suprarenal substance. It seemed desirable, therefore, to see if any definite objective evidence could be obtained that suprarenal substance when given by mouth has any specific effect in the body.

As further basis for the research there is on record some rather convincing evidence of a specific relationship between the suprarenal cortex and the sex glands. This evidence has recently been reviewed thoroughly by Glynn.[3] More recently McAuliff[4] has also briefly considered it.

That there is a relation between the adrenals and the sex glands was postulated by Meckel[5] as early as 1806. His conclusion was based on personal observations that in certain aborted fetuses both adrenals and gonads were lacking; in animals such as the guinea-pig, in which reproductive proclivities are marked, the adrenals are relatively large; in many animals the gonads and adrenals are closely associated in position, and in several cases of diseases of the genitalia adrenal degen-

* Submitted for publication Feb. 17, 1916.
* From the Laboratory of Physiology, Northwestern University Medical School.

1. Williams, T. A.: The Syndrome of Adrenal Insufficiency, Jour. Am. Med. Assn., 1914, lxiii, 2203.
2. Belfield, W. T.: Some Causes of Sterility and Impotence in the Male, *Ibid*, 1912, lix, 1420.
3. Glynn: Quart. Jour. Med., 1912, v, 157.
4. McAuliff, G. R.: Hypertrichosis, Variations in Female Secondary Sexual Characteristics, and Internal Secretions, Jour. Am. Med. Assn., 1916, lxvi, 15.
5. Meckel: Cited by Nagle, Note 6.

eration occurred. Meckel's evidence has since been controverted by various writers, notably by Nagle[6] in 1836, but the idea has persisted. Von Neugebauer[7] in 1908 was able to find in the literature thirteen cases in which hermaphroditism was associated with marked hypertrophy of the adrenals. Bullock and Sequeira[8] collected reports of twelve children showing sexual precocity who at necropsy proved to have enlarged adrenals. Various other instances of an association of sex anomalies with adrenal hypertrophy are on record.[4] The relation of suprarenal *deficiency* to the reproductive function seems to have received little attention. One of us, however, has observed a diminution in the number of offspring from female guinea-pigs deprived of one adrenal,[9] although the animals appeared to be perfectly normal otherwise.

The experiments on which this paper is based were made on white rats. The series comprises forty-eight experimental and twenty-five control animals. White rats were selected as subjects because of the ease with which they can be obtained and reared under laboratory conditions, and because the anatomical features of these animals have already been elaborately studied.[10] It was hoped that by duplicating the environmental conditions maintained by previous observers we should be able to use their results as a basis of comparison and escape the necessity of working out separate controls for our own series. This plan, however, proved impracticable. A preliminary investigation showed that our normal animals varied widely from the established "standards."

METHOD OF PROCEDURE

The following procedures were employed: The rats were bred in the laboratory. Of each litter one to three individuals were selected as controls and the others were put in the experimental series. They were weaned at the age of 16 to 24 days, and one to three days later the experimental feeding began. All members of the litter received the same treatment throughout, except that the experimental animals received desiccated suprarenal gland. (The commercial product of Parke, Davis & Co. was used.) It was first necessary to determine the proper dosage. The plan was to use as much as feasible. It was found, however, that the rats at first could not safely be given more than ¼ grain. This they received in the morning before being fed. The material was secured in 1 grain tablets. These were soaked in water until dissolved, then mixed with bread and milk. The members of each litter received their quota together. The food was scattered so that each got its proper share. It was eaten eagerly. As the animals grew older the size of the dose was increased, so that by the time the rat reached a weight of 100 gm. it was receiving from 1 to 2 grains. The material was administered once a day, six days a week. The experimental

6. Nagle: Müller's Arch., 1836, p. 365.
7. Von Neugebaur: Hermaphroditismus beim Menschen, Leipsic, 1908, p. 688.
8. Bullock and Sequeira: Trans. Path. Soc., London, 1905, lv, 189.
9. Hoskins, R. G.: Am. Jour. Med. Sc., 1911, cxli, 374, 535.
10. Donaldson: The Rat; Memoirs Wistar Inst. No. 6, Philadelphia, 1915.

period varied from two to eight weeks. The feeding continued in each case up to the time of sacrificing the animal.

Since the animals were to be killed before the breeding age, the sexes were not segregated. In case of none of the animals was copulation observed, nor did any pregnancy occur. They were fed cracked corn and bread and whole milk. The corn was kept constantly before them and they received all the bread and milk they would eat once a day. In some cases the control animals received enough dried blood to compensate for the protein of the suprarenal substance fed. In other instances this was omitted. In view of the fact that each rat was on full milk diet, the food value of the small quantity of desiccated gland was deemed negligible.

At various ages from 5 to 12 weeks the animals were killed. They were weighed in the morning before receiving the bread and milk. Then, having been chloroformed, each was bled to death from the aorta or heart. The following glands were at once removed and weighed: Hypophysis, thyroid, thymus, heart, suprarenals, spleen, kidneys and ovaries or testes (freed from epididymus). Each organ was carefully freed from adventitious tissues and rinsed in Ringer's solution. It was then dried on filter paper and quickly weighed on a delicate balance. The hypophysis and ovaries were weighed to tenths of a milligram and the other organs to milligrams or centigrams, depending on the size. For details as to securing the different organs the reader is referred to Jackson's descriptions.[11] The smaller organs can best be isolated by dissecting them out freely and transferring to a piece of smooth cork. The organs are rolled about the adventitious tags of tissue which adhere somewhat to the cork can be trimmed off with a sharp scalpel. A dissecting microscope is needed at first, but with practice this can be dispensed with.

The data secured were studied as follows: The organ weights were first reduced to percentages of body weights. These percentage weights were then transferred to coordinate paper, using the percentage weights as ordinates and the body weights as abscissae. A homogeneous distribution of the dots representing experimental and control weights was interpreted as showing that the suprarenal feeding had had no significant effect on the growth of the gland in question. This in turn was taken to indicate that no significant influence on the gland function had been exerted. A segregation of dots, on the other hand, indicated a corresponding influence on the gland. The thymus gland is supposed to vary according to age rather than to body weight. We plotted the percentage weight of this organ, therefore, against both body weight and age.

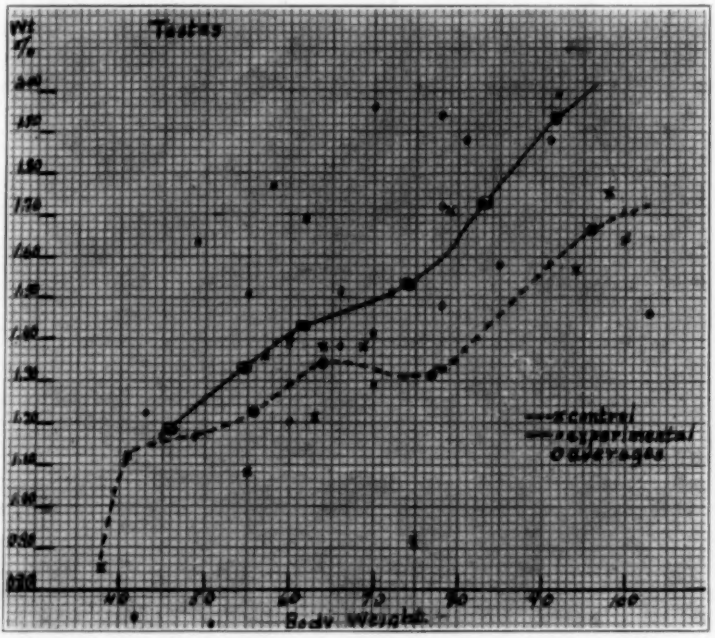
RESULTS

In case of most of the organs, no consistent deviations from the normal could be detected. The relatively small number of animals used as compared with the variability encountered would not permit the recognition of smaller deviations, even though these were characteristic. Our negative results are therefore not conclusive. As regards the pituitary, and, in case of the smaller animals, the ovaries, our method of weighing seemed inadequate. Evaporation might easily have introduced significant errors. The spleens of the experimental series averaged somewhat smaller than those of the controls, but the spleen is an unusually variable structure. This result, therefore, is only suggestive. The ovaries of the sixteen experimental animals were larger

11. Jackson: Am. Jour. Anat., 1913, xv, 1.

than those of eight controls, but these numbers are too small to mean much. They justify further study, however.

In case of the testes, a rather consistent hypertrophy was observed. The chart is reproduced in the accompanying figure. The table includes the data on which the chart is based. Even disregarding the extreme cases, which are of questionable significance, the average of the experimental animals is materially higher than that of the controls. This series comprised twenty-six and thirteen animals, respectively.



Weight of testes of thirteen normal rats and twenty-six rats fed with desiccated suprarenal gland. Ordinates = gland weights expressed as percentages of body weight; abscissae = body weight.

The influence of the suprarenal feeding on body growth was also considered. There was no difference that could be detected. There is much variability in growth, however, even within a given litter kept under identical conditions, and this would render the detection of slight differences impossible in a series of this magnitude.

The study is obviously incomplete. It is regarded as merely a preliminary investigation. It should be extended to include a larger number of individuals and different species. Careful cytological study should be made of the testes to see whether the spermatogenic or the interstitial tissue is affected, or if both share in the hypertrophy. Cor-

related with this, an investigation should be made of the effects of suprarenal feeding on the development of the sex characteristics. For such a study some animal, such as the chick, with well-marked secon-

TABLE OF WEIGHTS EXPRESSED AS PERCENTAGES OF BODY WEIGHT OF TESTES OF NORMAL AND EXPERIMENTAL WHITE RATS AT VARIOUS AGES, THE LATTER FED DESICCATED SUPRARENAL GLAND

Experimental				Control		
Body Weight, Gm.	Age, Days	Days Fed	Testes Weight, Per Cent.	Body Weight, Gm.	Age, Fed	Testes Weight, Per Cent.
				38	30	0.85
42	31	14	0.73			
43	63	41	1.21			
45	39	17	1.16			
49	56	34	1.63			
49	43	21	1.17	41	56	1.12
Av. 45.8	46.4	25.4	1.18	41	56	1.12
51	63	46	0.71			
55	43	21	1.51	55	29	1.08
58	43	21	1.77	57	43	1.36
Av. 54.7	51	29	1.33	56	41	1.22
60	41	19	1.20			
60	43	24	1.29	60	82	1.40
62	43	24	1.69	63	43	1.21
66	64	39	1.51	64	43	1.38
66	42	20	1.38	60	41	1.38
Av. 62.8	46.6	25.2	1.43	64	52	1.34
70	42	24	1.29			
70	64	39	1.42			
70	68	46	1.56			
72	65	43	1.51			
75	65	42	0.91			
78	82	56	1.48			
78	82	57	1.72	75	81	0.92
78	61	34	1.94	79	64	1.71
Av. 74	66.2	42.6	1.53	77	72.5	1.32
81	81	55	1.58			
85	82	56	1.58			
Av. 83	81.5	55.5	1.73			
91	78	57	1.88	94	79	1.57
92	66	39	1.99	98	86	1.75
Av. 91.5	73	48	1.93	96	82.5	1.66
103	79	56	1.46	100	66	1.64

dary sex characteristics, might be more satisfactory than the rat. As one phase of such a study the age at which breeding begins should be considered. These points we hope to investigate later.

The question arises: To what constituent of the gland material is the testicular hypertrophy due? In the clinical reports of suprarenal hypertrophy associated with sex anomalies the cortex of the gland mainly is affected. The amount of medullary material, namely, adrenin, in the small quantity of gland substance fed, was of course minute. Moreover, absorption of adrenin from the gastro-intestinal tract occurs only to a slight extent before the material is destroyed. It is altogether likely, therefore, that the hypertrophy observed is to be ascribed to the cortex portion.

Possibly the results secured would justify further attention to the therapeutic possibilities of desiccated suprarenal in cases of infantilism or sexual impotency. Often more than one of the endocrinous glands are involved, and in such cases no one gland substance could be expected to restore the balance. In cases, however, in which the fault lies primarily with the suprarenals, such medication might be effective.

SUMMARY AND CONCLUSIONS

Forty-five white rats were fed desiccated suprarenal for varying periods from two to nine weeks. Twenty-six animals from the same litters were kept as controls. The rate of growth and the weights of various glands were determined in each series. No differences in the two series could be detected in kidneys, heart, hypophysis, thyroid, thymus or suprarenals. The spleens of the experimental series were somewhat smaller than those of the controls, but highly variable. The ovaries in the few cases studied were larger in the experimental series. The testes (twenty-six experimental, thirteen control) showed hypertrophy. These results in confirmation of clinical evidence indicate that the suprarenals exert a stimulating effect on the testes.

2431 South Dearborn Street.

BOOK REVIEW

PATHOGENIC MICRO-ORGANISMS. (Including Bacteria and Protozoa.) A Practical Manual for Students, Physicians and Health Officers. By WILLIAM H. PARK, M.D., Professor of Bacteriology and Hygiene in the University and Bellevue Hospital Medical College, and Director of the Bureau of Laboratories of the Department of Health, New York City, and ANNA W. WILLIAMS, M.D., Assistant Director of the Bureau of Laboratories, New York City; Consulting Pathologist to the New York Infirmary for Women and Children. New (5th) edition, thoroughly revised. Octavo, 684 pages, with 210 illustrations and 9 full-page plates. Cloth, $4.00, net. Lea & Febiger, Publishers, Philadelphia and New York, 1914.

The fifth edition of this authoritative work is well adapted to continue its popularity. It has been revised thoroughly, new material has been added in many places, and some rearrangements have been made that improve its order and add to the ease of reference. As an example of completeness of revision may be mentioned the description of the Schick reaction for the detection of immunity in diphtheria, and its technic. The account of the bacteriology of pneumonia is also as near the times as possible. At the same time, the rapidity of progress in bacteriology is illustrated by the fact that the important work on *Monilia* by Ashford and others appeared after the publication, while most of the work on the endamebas of the mouth was too recent to be included. In the discussion of streptococci, which is very good, more could have been said of septic endocarditis. There are reasons for thinking many practicing physicians do not keep as well informed on all the phases of microbiology as they should. For these, original articles are too numerous and too hard to understand. This work, by reason of its concise and yet lively style, and the unusual experience of its authors as investigators as well as clinical and public health experts, can be consulted with satisfaction by all classes of readers—beginners in bacteriology, professionals in boards of health, and practitioners of medicine in all its branches, and in all degrees of bacteriologic preparedness or unpreparedness.

The mechanical execution of the book is good on the whole, but many of the halftone and color illustrations are sadly inferior. The bibliographic references are well selected, but the spelling of proper names has suffered rather more than it should. It is to be hoped the work will continue to receive revision and retain its well earned reputation.